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STUDIES ON ELEMENTAL SULFUR AS A SOIL INSECTICIDE.*

JACOB WORK BULGER

Assistant Agricultural Physiologist,
Bee Culture Laboratory, Somerset, Maryland.

INTRODUCTION.

Soil insects have always been a very serious problem to those engaged in agriculture. Numerous insects, chiefly in their larval form, almost universally infest both virgin and cultivated soils. The groups most commonly troublesome are grubs, wireworms, cutworms, rootmaggots, and rootaphids, or root lice, as they are commonly called. Certain species of a few other groups also cause, at times, considerable injury.

It would hardly seem necessary to emphasize the economic importance of each of these forms. The common white grub alone causes annually thousands of dollars of loss in agricultural products. Davis (*2*†) estimates that in 1912 this pest alone caused a loss of approximately $7,000,000 to corn, timothy, and potatoes, in the states of Iowa, Illinois, and Wisconsin. He also estimates the aggregate damage from this one insect throughout the entire country in the year 1912 to be upwards of $12,000,000. It is doubtful if adequate data are available to make anything like an exact evaluation of the loss in agricultural products sustained annually through soil-feeding insects. It would seem to the writer conservative to assert that throughout the entire country soil insects cause a loss of many millions of dollars annually.

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* Presented in the graduate school of the Ohio State University in 1924, prior to the author's appointment to a position in the Bureau of Entomology, United States Department of Agriculture, Washington, D. C., in partial fulfillment for the degree of doctor of philosophy.

† Figures (italics) in parentheses refer to the bibliography.

In recent years much work has been done along the line of insect control. Much more progress has been made in the control of air-feeding forms than of those that spend most of their feeding period in the soil. At the present time most insecticides are very limited in their use against soil-feeding insects, though some of them are very effective against forms which feed on leaves. This is probably because the conditions under which insecticides must be used in combating soil insects are so different from those under which they are applied in the control of forms that feed upon the aerial portions of plants that an extensive or general use of most known insecticides is impracticable, if not almost impossible. This is particularly true in the case of stomach and contact poisons. Volatile or gaseous insecticides are also limited in their use as soil insecticides but it would seem to the writer that they have more promise as general soil insecticides than either stomach or contact poisons.

The ideal soil insecticide would be some substance which, when applied to the soil, would exert a toxicity fatal to the insect fauna which inhabit it, and at the same time would not produce any permanently detrimental effect on cultivated crops. It is not probable that any such a panacea will soon be discovered. However, if some substance of use in agriculture could be found effective as an insecticide, even to a limited extent, or if such a substance was found capable of being manipulated in such a manner as to give it insecticidal value, the dual value of such a substance would aid materially in promoting its use as a soil insecticide. It was with this idea in mind that the writer undertook the study of the insecticidal properties of elemental sulfur.

Elemental sulfur has been found to have value as a fertilizer, chiefly in some of the western states. It has been found effective in the control of potato scab, and useful in producing a reaction favorable to crop growth in alkali soils. There are also some unauthenticated rumors as to its value in controling insects in places where it was used to control potato scab.

Investigations as to the chemical reactions of sulfur, which will be taken up in detail later in this paper, show that sulfur in the soil is converted to sulfates. It is known that during this process sulfurous and sulfuric acids are formed. In the early stages of this process, at least theoretically, SO_2 is produced, and under restricted conditions (the breaking down of

organic matter, especially that rich in protein) H_2S may be produced. If some of these transitional products, or some other products resulting from the reaction of sulfur with certain soil elements, should be found toxic to soil insects to an extent sufficient to make them of insecticidal importance, the fertilizer and other previously mentioned used of sulfur in the soil in many cases might aid in making its use as a soil insecticide more practical.

The object of this research, and the purpose of the Texas Gulf Sulfur Company and the National Research Council in initiation and supporting it, was to ascertain the possibilities and the limitations of elemental sulfur as a soil insecticide. In this work, therefore, the investigator was limited to the use of elemental forms of sulfur. Inoculated sulfur and flour of sulfur were the two forms uses in most of the experiments of this research. A more complete statement concerning them is given later in this paper.

The term "Soil Insect" is used in this paper only in referring to those insects the adult or larva of which spends a considerable portion of its feeding period in the soil.

In the selection of insects for use in this research it was thought advisable to experiment on forms representing as widely differing types as were available, and, at the same time, forms which were of great economic importance. With this in mind the writer chose ants (*Formica fusca subsericea*), root maggots (*Hylemyia fusciceps, and H. Brassicea*), the common white grub (*Phyllophaga Sp.*), wireworms, rootaphids, and cutworms.

In some preliminary work the rootaphis was found not to lend itself very readily to experimentation, and consequently very little work was done with it. A brief discussion and reference to some work done by the author in conjunction with Dr. C. R. Cutright, of the Ohio Experiment Station, Wooster, Ohio, is given in the latter part of this paper.

Two general types of experiments were carried on in this research: (a) insectary tests to determine the insecticidal possibilities and limitations of elemental sulfur and some of its transitional products, such as SO_2, H_2SO_3, H_2SO_4, and H_2S, which, according to Joffe (*4*), are produced in the soil through the transformation of sulfur to sulfates; and (b) field-plot experiments, in which observations were made as to the degree of injury done by insects to crops grown on plots the soils of which had been treated with elemental sulfur. Observations

were also made during these experiments on the effect of sulfur treatments upon the soil and the vegetation supported by it.

The Transformation of Sulfur in the Soil.

The transformation of sulfur in the soil may take place either by oxidation or by reduction. The end product of either of the processes is a sulfate, which is the form in which sulfur is utilized by plants. It is evident then that through its transformation into sulfates, sulfur is made available to plants.

Sulfur transformation in the soil occurs chiefly through oxidation. However, under anaerobic conditions such as are found in the organic muck at the bottom of lakes, or in any place where vegetative or organic decay takes place, largely in the absence of air, sulfur may be reduced and H_2S be liberated. This in turn is oxidized, and sulfates are ultimately formed as in the case of sulfur oxidation.

From Joffe's review of the literature (*4*), which deals with the transformation and uses of elemental sulfur, there seems to have been considerable controversy between some of the early workers as to whether sulfur transformation was a chemical or a biological process. Recent work in which special sulfur-oxidizing bacteria have been isolated has established quite conclusively that this transformation is, at least, largely biological.

A number of organisms capable of oxidizing and some capable of reducing, sulfur have been isolated. The most important of these sulfur-transforming organisms are two species which oxidize sulfur:—*Thiobaccillus thioparus*, Nathanson (*7*); and *Thiobaccillus thiooxidans*, Lipman, Waksman, and Joffe (*8*). According to Joffe (*4*) it is doubtful if T. thioparus is capable of oxidizing elemental sulfur, although Jacobsen (*3*) reports that it is. Joffe points out that these two organisms have very fundamental physiological differences, the latter being very active in alkaline, and the former in acid, mediums.

In carrying out this research the writer is indebted to Dr. A. G. McCall, chairman of the Salt Requirement Committee of the National Research Council, for the fellowship through which the work was made possible and for the assignment of the problem; to the Texas Gulf Sulfur Company, through Dr. Clayton H. Lint, for funds supporting the fellowship and for sulfur used in the various experiments; to Dr. Herbert Osborn, of the Department of Zoology and Entomology of Ohio State University, for his many helpful suggestions and general supervision of the problem; to the Department of Zoology and Entomology of Ohio State University for equipment and materials; to various members of the University staff for suggestions and advice from time to time; and to Mrs. J. W. Bulger for her constant encouragement and help.

The oxidation of elemental sulfur, whether chemical or biological or both, results in the production of sulfuric acid which in turn reacts with various soil constituents to form sulfates. Soils that are highly buffered may not become acid for some time. In many soils, however, especially those that have been farmed for a considerable time, the oxidation of sulfur changes the reaction rapidly so that the soil soon becomes strongly acid.

Reactions for sulfur oxidation in the soil

Joffe (*4, p. 48*) gives the following reaction for the oxidation of sulfur in sulfur-float mixtures:

$$2S + 3O + 2H_2O = 2H_2SO_4$$

This reaction is probably identical with that which occurs in a soil which has been treated with elemental sulfur. Let us now follow step by step what probably occurs in this reaction. If we begin with elemental sulfur we may assume that, at least momentarily, SO_2 is produced as the first product of sulfur oxidation:

$$S + O_2 = SO_2$$

When SO_2 comes in contact with the moisture in the soil it almost immediately unites with a molecule of water and forms sulfurous acid:

$$SO_2 + H_2O = H_2SO_3$$

This in turn undergoes oxidation and sulfuric acid is produced:

$$6H_2SO_3 + 3O_2 = 6H_2SO_4$$

From this analysis of the reactions taking place in the oxidation of sulfur it would appear that when the oxidation of sulfur occurs in a soil SO_2, H_2SO_3, and H_2SO_4, all of which might be toxic to soil insects, are produced. Under special conditions, as previously noted, H_2S might also be produced in the course of the transformation of sulfur. The experiments described in the following pages were undertaken to determine the toxicity of these transitional products of sulfur to some forms of a few soil insects.

General Experimental Procedure.

Types of Experiments.

In the carrying out of this research both insectary and field methods were used. In the insectary tests were made to determine the toxicity of the previously mentioned products of oxidation and reduction of sulfur to soil insects, as well as any direct effect that elemental sulfur itself might have upon them. In the field turnips, radishes, and potatoes were grown on soils treated with sulfur and the degree of infestation of crops by rootmaggots and white grubs noted. The detailed results of these experiments will be given further on in this paper.

Kinds of Sulfur Used.

As previously stated one of the specific conditions of the fellowship fostering this research was that it should be confined to the use of elemental sulfur, thus excluding the use of any of the commercially prepared compounds of sulfur except where they might be used in conjunction with some elemental form of it, as, for example, in experiments where sulfur was tested as a carrier of materials of known insecticidal value, such as carbon bisulfide or nicotine sulfate.

In the various experiments of this research only flour of sulfur and inoculated sulfur were used. These were chosen because they are the principal forms of elemental sulfur used in work relating to soil fertility and to the control of potato scab. Both of these products were furnished by the Texas Gulf Sulfur Company. Flour of sulfur is simply finely ground brimstone, and inoculated sulfur is flour of sulfur which has been inoculated with a species of bacteria known to be especially active in the oxidation of sulfur. A few preliminary tests were made in which the activity of flour of sulfur and flowers of sulfur were compared, and since no difference in reactivity could be detected, the latter was not used in any of the tests recorded in the following pages.

The Determination of Soil Acidity.

Since soil acidity is one of the results of the oxidation of sulfur in the soil, and because this acidity might have some effect upon the insect fauna of the soil, it was necessary to determine the acidity of each of the soils in the following experiments and to note it in connection with insecticidal and other observations.

Although various acids may be equal quantitatively, they usually do not dissociate equally; in other words they do not possess equal intensities. It is therefore evident, so far as biological effect is concerned, that a method of determining acidity, which is a measure of the intensity, without regard to which or how many acids are present, is the most suitable. For this reason the method of determining the hydrogen-ion concentration, which Clark (*1*) has termed the method of measuring the intensity of an acid, was used in preference to measurements involving titration with standard alkali solutions, which are really only quantitative determinations.

The Determination of Hydrogen-ion Concentrations.

There are two general methods by which hydrogen-ion determinations are commonly made; first, the electrometric method, which is really the basis for all hydrogen-ion measurements, and when carefully and properly made gives much more refined results than any other method; second, the colorimetric method, (*5*), by means of which measurements can be made directly to within 0.2 of a pH, and by interpolation values as small as 0.1 pH can readily be made.

Although the electrometric method is much the more refined, and even makes possible the making of measurements in cases where physical and chemical factors would interfere with the accuracy of colorimetric tests, the technique necessary for the proper manipulation of an electrometric apparatus is greater, while the convenience and rapidity of making the tests is less, than those of the colorimetric method. The cost of a reliable and convenient electrometric apparatus is also greater than that of a colorimetric one. For these reasons the colorimetric method was chosen and used in this research in making the determination of pH values.

Apparatus For Colorimetric Hydrogen-ion Determinations.

For the determination of hydrogen-ion concentrations the following are necessary: indicators (Clark *1*–, p. 80); standard buffer solutions from which a series of solutions, ranging in pH value from 1.2 to 9.8, can be prepared; about a dozen test tubes of approximately 20 c. c. capacity and of uniform diameter, so that the same depth of liquid will be concerned in all comparisons; and some wide-mouthed glass bottles in which to prepare extracts of the substances the hydrogen-ion concentrations of which are to be determined. The buffer solutions recommended by Clark (*1*) are very satisfactory: in this research, however, those recommended by MacIlvaine (*6*) were found equally satisfactory.

Samples For Use in Hydrogen-ion Determinations.

For each hydrogen-ion determination from 2 to 10 grams of soil were taken, placed in a clean glass bottle, and set aside until a number of samples had been collected. Generally the determinations were made within from 24 to 48 hours after the samples had been taken.

In the insectary experiments, where the sulfur and other constituents were more or less intimately mixed with the soil, only one sample was taken from each container. This sample was taken at the time when the soil had been removed from the various containers, so that observations could be made as to the condition of the insect. Each of these soils was thoroughly mixed before the sample was taken and then the soil and insect were again placed in the container.

In the field experiments determinations of hydrogen-ion concentration were made both for the surface (0 to 2 inches deep) and for the sub-surface (2 to 4 inches deep). In procuring samples for these determiantions samplings were made at three or four representative places on each plot. After all the samples which were to be taken on a particular date had been procured the hydrogen-ion concentrations of all were determined in accordance with the details given below.

Procedure of Making Hydrogen-ion Determinations.

During most of the year 1922-23, through the courtesy of the Department of Physical Chemistry of Ohio State University, the determination of hydrogen-ion concentrations were made in the above laboratory by means of indicators and buffer solutions which they had prepared in accordance with the method of Clark (*1*). At beginning of the year 1923-24, on account of the inconvenience of this procedure and the time required to transport all the solutions to and from this laboratory, dyes were procured and a set of indicators prepared according to Clark (*1*, p. 80, 81). The buffer solutions used with this set of dyes were prepared according to MacIlvaine (*6*) instead of according to Clark. This change was made because by means of these buffers a satisfactory color range could be prepared with but two solutions, small portions of the two being united in such a manner as to procure a range of pH values from 2.0 to 8.0.

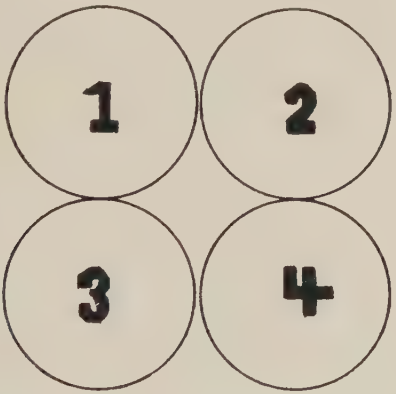
In the experiments described in the following pages it was not found necessary to make measurements of pH values above the upper limit of this range. Where it was desirable to make measurements of acidity represented by pH values below the lower limit of this range, the buffer solutions of the Department of Physical Chemistry of Ohio State University were used.

Manipulations in the H-ion Tests.

In making the various determinations of pH value each sample of soil was thoroughly mixed and the coarse sand and pebbles removed by means of a 12 mesh wire screen; 2 grams of this soil were then placed in a clean bottle of approximately 100 c. c. capacity and 100 c. c. of distilled water was then added; the contents of the bottle were vigorously shaken and then set aside until the solution became clear enough to make color comparisons with standard buffer solutions colored by the same indicator. In from 2 to 4 hours the solutions were usually clear enough to be fairly workable. It was found by preliminary tests that leaving a solution for this length of time did not usually cause any significant change in its pH value.

The approximate pH value of the solution being tested was first determined by placing about 2 c. c. of the clear solution in a test tube and adding a drop of the indicator within the range of which it was thought the pH value of the solution in question might fall. A glance at this test enables one to see if the proper indicator has been used and, if not, whether the solution is more or less acid than can be determined by it. If the first indicator tried is not the proper one, other indicators, selected in accordance to the indications of this test, are used with similar portions of the unknown solution, until one is found with which this particular determination can be made. Next, 10 c. c. of the unknown are pipetted into a test tube and 5 drops of the indicator then added. After shaking, so as to distribute the color uniformly throughout the solution, color comparisons were made with tubes containing the same quality of buffer solution which had been colored with the same indicator. The pH value could then be read from the standard buffer tube whose color most nearly matched that of the unknown solution.

Even after 4 hours or longer, the cloudiness of the solutions made the determinations difficult when compared in the usual manner with the colored standard buffer solutions. A simple method of holding the tubes, suggested by Dr. Thomas G. Phillips, of the Department of Agricultural Chemistry of Ohio State University, and illustrated in the accompanying diagram, helped very materially in getting more nearly the correct readings. In the diagram 1 is a water blank, 2 a tube of the unknown solution to which no indicator has been added, 3 is precisely like 2 except that it contains five drops of the appropriate indicator, and 4 is a tube of the buffer solution to which five drops of the indicator has been added. By arranging these tubes as shown in the diagram, holding them up to the light, and placing different standard buffer tubes in position 4 until one is found which matches the color of the unknown to which the indicator had been added, it was a fairly easy matter to determine the pH value of the somewhat turbid solutions.



In all the hydrogen-ion tests made in the spring and summer of 1924, which include a large portion of the experiments the data of which are given in this dissertation, the solutions were centrifuged free of sediment before the hydrogen-ion determinations were made. This procedure was much more satisfactory, and greatly facilitated the determinations.

Feeding Insects in Insectary Experiments.

Where the soil acidity was great, the feeding of insects in insectary experiments was rather difficult on account of the effect which this acidity had upon the green vegetation, roots, seeds, etc., that were used as food.

Where the soil was not too acid for seed germination and plant growth, corn seedlings were used as food. In the more acid soils pieces of potatoes, fresh grass, grass roots, etc., were used instead of corn. These materials were placed in the soils of the various containers and renewed from time to time in the course of each experiment, so that a supply of fairly fresh food was always available. In the insectary experiments in which direct treatments of sulfur were made, the same

general method of feeding was followed, and the same food materials were used.

In all experiments upon ants granulated sugar was used as food. A small tin or paper tray containing a small quantity of sugar was placed upon the surface of the soil of each nest. The sugar was replenished from time to time, so that a constant supply of food was always available.

Moisture Content of Soils Used in Insectary Experiments.

All the soils used in the insectary experiments were first reduced to an air-dry condition. Stocks of the soils used in making up the various tests were kept in the insectary in that condition, so that tests could be prepared with a definite, known content of water, thus making it possible to keep the moisture content of each soil approximately constant throughout the course of each series of experiments.

The amount of moisture which it was considered desirable to maintain throughout most of the insectary tests was that which would be about the optimum for plant growth. This was determined by a method, recommended by the Department of Soils of Ohio State University, which consists in making a soil sufficiently moist that when grasped in the hand it would cohere just enough to form a slight ball. The quantity of water necessary to make of this consistency the soil, from which the stock soils used in the various insectary experiments were prepared, was calculated as 0.4 c. c. per gram of air-dry soil. This quantity of water was added to the stock soil in the preparation of each series of experiments except those in which tests for the production of H_2S were made.

The moisture content of the soils of each series of experiments was kept as constant as possible throughout the course of each experiment, by frequent weighings and the addition of water when needed. This eliminated as far as possible the effect that a variation in moisture content might have upon the insects.

In the experiments where toxicity of H_2SO_2 and H_3SO_4 to insects was being tested, the quantity of water required daily was omitted for several days before the acids were applied, or until about 100 c. c. of water was required to make the soil of each container up to its required moisture content. This made it possible to add the acid with the water and in this manner to obtain better and more immediate distribution of the acid throughout the soil, and at the same time to avoid adding more liquid than would just make each soil up to the desired weight.

The Preparation of Acid Soils For Use in Insectary Experiments.

Since sulfuric acid is known to be the chief acid which is produced in the soil through the oxidation of sulfur, in order to obtain more quickly soils with the desired degree of acidity, pots of field soils, with pH values of about 6.8 to 7.2, were treated with varying quantities of sulfuric acid until, when the reaction had reached an equilibrium, 6 soils had been obtained with pH values of approximately 2.0, 2.3, 3.4, 4.7, 5.1, and 7.0, respectively. These soils were then air dried and a supply of each kept in this condition in the insectary as stocks from which to prepare the various series of acid soils used in the toxicity experiments noted in the following pages.

Temperature Regulation in the Insectary Experiments.

No device for controling the temperature of the soils in the various experiments was available, and so the pots and cages used in each experiment were simply placed in as nearly identical conditions of temperature as were possible. By this procedure it was thought that the temperature of the various soils of each series would at least be comparable.

Toxicity to Ants of Some Products of Sulfur Oxidation and Reduction.

Since H_2SO_4 is known to be produced in the soil through the oxidation of sulfur and since it is probable that SO_2, H_2SO_3, and possibly H_2S are produced, under certain conditions, in the course of this reaction, it was thought advisable to ascertain the degree of toxicity of each of these products to some form of an insect which lived in the soil.

Ants (*Formica fusca subsericea*) seemed to thrive fairly well under cage conditions, and for that reason were chosen as the insect to be used in these tests. Cages with tightly fitted lids of screen, Plate 1, B, were used to confine the ants so that definite observations and counts could be made.

Tests were simultaneously made with the first three of the substances named, and the details and results are presented in Table I. Equal quantities of air-dry soil were placed in the several containers and then made up to and maintained at a uniform moisture content. The same number of worker ants were placed in each cage, and the various substances applied after the ants had established nests.

The reaction of the soil was recorded in terms of pH value which was determined from a sample taken when the soil was removed to make counts of the number of living ants and to thoroughly mix the soil in order that it might have a uniform reaction throughout. Further details are given in the discussion of the treatment by each specific substance.

Sulfur Dioxide.

Theoretically, SO_2 is the first product formed in the oxidation of sulfur in the soil. Though it almost immediately unites with the H_2O of the soil and forms sulfurous acid, it was thought that possibly during this reaction SO_2 might be of some insecticidal importance. For this reason an experiment

was planned (cage 20, Table I) to determine if it is possible to introduce enough SO_2 into a soil to be of value insecticidally.

By means of hard rubber and glass tubing, SO_2 gas was bubbled into the soil of the cage from a cylinder of compressed gas and it was distributed throughout the soil by means of perforations in that portion of the rubber tubing which was in the soil. This portion of the tube was 6 or 7 inches long and was coiled at the bottom against one side of the battery jar. The tube was placed in this manner, rather than entirely encircling the jar, in order that a better idea might be obtained

TABLE I.

DATA RELATING TO EXPERIMENTS ON THE TOXICITY OF SO_2, H_2SO_3, AND H_2SO_4 TO ANTS (*Formica fusca subsericea*).

Cage No.	Number of ants introduced May 5th	Soil (b) treatments May 7th		Living Ants May 15	Ave. pH Value of	Observations May 23rd
		Material	Quantity c. c.			
18	20	H_2SO_4	40	0	2.55	.....
19	24	H_2SO_4	25	8	4.8	Very little activity.
20	20	SO_2	(a)	9	7.1	Ants active.
21	20	No treatment	.....	18	7.4	Ants very active.
22	21	H_2SO_3	40	8	7.3	Some activity.

(a) This gas was bubbled into the soil for several hours from a cylinder of the compressed gas at the rate of 40 bubbles per minute.

(b) The soil of each nest consisted of 800 grams of air-dry soil and 400 c. c. of tap water.

as to the penetrating ability of the gas. After it had been placed in position the ants were put into the cage and allowed to establish their nest before introducing the gas.

In order to obtain some idea as to how rapidly the gas was being forced into the soil the end of the tube connecting the cylinder with the distributing tube was held under water and the number of bubbles per minute counted, before it was connected with the tube in the cage. At the beginning of the experiment, 4 P. M., the jet was regulated at 40 bubbles per minute; at 7 P. M. the gas was found to be running at the rate of only 4 bubbles per minute. It was again adjusted at 40 per minute and left undisturbed until morning, when it was found that the pressure had decreased enough that the bubbling had ceased though the tank still had gas in it.

After 8 days nine of the ants were still living. In this time 11 dead ants had been removed from the surface of the soil, most of the having been found directly over the perforated part of the discharge tube.

It is evident that in this experiment SO_2 did not give a very satisfactory insecticidal effect, even when bubbled into the soil at a more rapid rate than one would expect the gas to be produced in a soil through the oxidation of sulfur. It is also evident, since most of the dead ants were found directly over the place where the gas was discharged into the soil, and the living ants were carrying on their activities toward the opposite side of the cage, that SO_2 does not penetrate far into a soil. This lack of penetration is more than likely due to the fact that it unites so readily with the H_2O of the soil, thereby forming sulfurous acid.

Experiments cited later in this paper, in which elemental sulfur was applied to the soil of field plots, indicate that the soil from which the soil used in this test was taken was very highly buffered, or at least, the application of as much as 4,000 pounds of sulfur per acre made only a slight change in its acidity.

While this test shows that SO_2, even when applied in large quantities, is not efficient as a soil insecticide, it also shows that this gas if present in the soil in a sufficient quantity, is considerably toxic to the ant, *Formica fusca subsericea.*

Sulfurous and Sulfuric Acids.

The application of sulfurous acid was made by pouring it into the soil, after the ants had established nests in soils prepared as indicated in Note b, Table I. In order to obtain better distribution of the acid throughout the soil, the addition of the quantity of water necessary to keep the soil at a constant weight was omitted until the deficit amounted to about 100 c. c. The quantity of acid indicated in Table I was then added to just enough water to make the number of c. c. of liquid added just equal to the deficit in moisture content.

With this method of procedure a better distribution of the acid throughout the soil was obtained and at the same time the liquid content of the soil was not increased beyond that previously determined as that to be maintained throughout the test. The applications of sulfuric acid were made in the same manner as that used in case of sufurous acid. In these tests 50 per cent commerical acid was used.

The soils, both in the sulfuric and sulfurous acid tests as well as in the check, were removed from time to time in the course of the experiments to permit definite counts of the number of living ants. At these times the soil of each was intimately mixed, so that its acidity would be more uniform throughout.

From Table I it will be seen that sulfurous acid was somewhat toxic to ants, even though the acidity of the soil was ultimately but slightly increased. The toxicity in this case might have been due to the fact that when the acid solution was poured upon the soil in the cage it run down the burrows, and probably came directly in contact with some of the ants. This, at least, was true in some of the nests which were treated with sulfuric acid. It is also probable that the soil along some of the burrows was much more acid than that farther from them, since the quantity of solution added was found insufficient to wet all the soil in the nest.

The results set forth in Table I show that sulfuric acid is somewhat toxic to these ants. Some ants, however, lived for several days in one of the soils treated with this acid. This was also true in some preliminary experiments in which the soil of ant nests was treated with sulfuric acid. In these preliminary experiments wooden cages with glass sides were used. The ants were induced, by keeping the sides of the cages covered with cardboard, to make their burrows out against the sides of the cage, thus enabling observations to be made upon them while in the burrows.

The treatment of the soil in this type of cage was much like that of cages 18 and 19 (Table I), except that the soil was not removed and mixed at the time the observations were made. In these preliminary experiments some ants lived and continued active for nearly 50 days and although no queen was present and no eggs were seen at the time the soil and ants were brought in from the field, several larvæ were reared to maturity and emerged as queens while these experiments were in progress.

The hydrogen-ion concentration of the soils in the cages of these preliminary experiments varied from 1.9 to 5.6 pH in the nine determinations that were made. This wide variation was undoubtedly due to the imperfect distribution of the acid throughout the soil. The average pH value was 3.0 for the soil of one cage, and 3.5 for the other. In these cages the samples used in making determinations of the pH value were

taken by means of a small auger, in such a way that a composite sample of the soils from top to bottom of the cage was obtained.

Although the results of this set of tests are not entirely conclusive, they indicate that sulfuric and sulfurous acids are toxic to ants under the conditions in which they are applied in these experiments. They also show that ants are able to endure very high degrees of soil acidity.

Hydrogen Sulfide.

In order to determine whether hydrogen sulfide was toxic to ants, (*Formica fusca subsericea*), H_2S gas was generated from ferrous sulfate in a hydrogen generator and bubbled into the soil of an ant nest. The same cage and the same type of apparatus was employed as that used in testing the toxicity of SO_2. Since it was impossible to control the rate at which the gas was generated, no estimate could be made as to the rapidity with which it entered the soil.

The generator was connected with the cage and left running for a few minutes, or until a very decided odor of H_2S was detected in the soil of the cage. By this method H_2S was found to be decidedly toxic to these ants, for it was necessary to bubble it through the soil for only a few minutes to kill all the ants in the nest. It is not probable, however, that this gas is ever produced in the soil in any such a quantity as was used in this test.

Since this gas is produced in the soil through bacterial decomposition of organic matter, it was decided to attempt its production from composts of manure, soil, and sulfur. Fresh horse manure, which had been piled out of doors for about two months, but had not been permitted to burn out, was mixed with varying proportions of soil and elemental sulfur, as shown in Table II; each mixture was placed in a battery jar, and a quantity of water, noted in the table, added. In most cases the water was sufficient to produce a water-logged condition in the mixture.

This experiment was begun May 6 and terminated July 20. The presence of H_2S was detected by means of small strips of paper moistened with lead acetate solution and placed in Petri dishes resting on the surface of the composts. The papers in all the jars except those numbered 2 and 6 were blackened slightly, showing the presence of H_2S. On June 14 a fresh

lead-acetate paper was placed in each Petri dish, and the time required for each to begin to blacken was noted.

By this series of tests it was shown that H_2S is produced in composts of manure, soil, and sulfur, and that a high organic content, or a high moisture content, is necessary to its production.

In order to determine whether the quantities of this gas produced in these composts were sufficient to be of insecticidal value, 9 to 10 ants were placed in each of the jars numbered 1, 2, and 5, and observations were made as to the length of time

TABLE II.

DATA RELATING TO THE PRODUCTION OF H_2S, IN COMPOSTS OF MANURE, SOIL, AND SULFUR.

Jar No.	Quantity of Manure	Quantity of Sulfur	Quantity of Air-Dry Soil	Quantity of Water Added	Days Until Lead-Acetate Paper Blackened After Exposure on—	
	Grams	Grams	Grams	c. c.	May 6	June 14
1	908	15	0	0	11	4
2	36	3	1211	605	(a)	(a)
3	908	15	0	454	19	8
4	36	3	1211	908	(a)	36
5	454	100	454	454	27	10
6	908 (b)	0	0	0	(a) (c)	(a)

(a) Where no figure for days is given there was no change in color.

(b) In jar No. 6 manure alone, in the same condition in which it was when brought into the insectary, was used as a check.

(c) Exposed May 25. No lead-acetate paper previously in the jar.

the ants lived. The nature of the composts made counts of the numbers of living ants impossible. However, after 4 days living ants were seen in all of the jars, from which the conclusion was reached that the quantity of H_2S evolved was not sufficient to be of insecticidal value.

TOLERANCE OF INSECTS TO ACID SOIL.

In testing the tolerance of insects to acid soil, stock soils which had been made acid by the use of sulfuric acid solutions were used. Various series of pot and cage soils with pH values ranging from about 1.9 to about 6.4 were prepared and used in these tests. Insects were placed in or upon the soils of the

various containers, and observations were made from time to time as to the presence and condition of the insects. In making these observations the soil of each pot was removed, and before being returned to the container it was thoroughly mixed and a sample taken for the determination of the hydrogen-ion concentration. This mixing helped to create a more uniform reaction throughout the soil of each cage, and thus necessitated the taking of but one sample from each soil for the determination of its pH value.

By this method of making observations it was possible to determine just how long the insect lived in each of the soils, and by comparing the longevity of the various individuals to get an idea as to whether acid soil is toxic to insects, and how

TABLE III.

Data Relating to Experiments on the Longevity of Ants in Acid Soil.

Cage No.	Number of Ants Introduced	pH Value	Number of Living Ants Found in Each Cage on the Following Dates				
	June 18	June 19	June 20	June 23	June 27	July 1	July 7
1	12	1.9	0	.....	.....	.....	.....
2	12	3.3	9	9	9	9	8
3	12	5.2	10	9	8	7	7
4	12	6.4	10	10	10	10	9

acid a soil they can endure. Ants (*Formica fusca subsericea*) and the common white grub (Phyllophaga Sp.) were the chief insects used in these experiments. A few wireworms and some cutworms were also placed in some of the soils in the course of the white-grub experiments.

Ants.

In this series of experiments 12 ants (*Formica fusca subsericea*) were, on June 18, placed in each of four cages. The soils of three of these cages had been treated on May 18 with solutions of sulfuric acid and on June 19, after having been thoroughly mixed, they gave the pH values given in Table III.

As shown in the table, all the ants in the most acid soil were dead within 24 hours after being put into the cage. In the other cages the longevity of the ants was nearly uniform.

In order to verify the result in cage No. 1, another lot consisting of 6 ants was placed upon the soil of this cage at 10 A. M., June 20; at 5 P. M. of the same day 3 of them were dead, and by the next morning all 6 were dead.

From both this and preceding experiments in which ant nests were treated with sulfuric acid, it is evident that the species of ant used can tolerate a high degree of acidity. Although soils with as great an acidity as that in cage No. 1, are very toxic to ants, it is evident from the record for cage No. 2, that ants can live for a considerable time in soils which are too acid for the growth of much vegetation.

Just how the acidity of the soil in cage No. 1 caused the death of the ants was not determined. Titrometric methods of determining the acidity of the soil of this cage showed it to be slightly greater than that represented by the pH value. It is possible that insufficient time for the reaction to have reached an equilibrium had elasped since this particular stock soil had received its last application of sulfuric acid, and that acid soil in this condition exerts a greater effect upon ants than that of a soil whose acidity is entirely measureable in terms of pH value. It may also be that some volatile substance was produced, or that death was due to the dehydrating effect of the sulfuric acid upon the ants.

In a test in which ants were placed in a screen tray just above the surface of the soil of this cage, thus preventing them from coming in contact with the soil, it was found that the killing was almost as rapid as when direct contact was permitted. It was found that the higher above the soil the tray was placed the slower was the killing. With the tray 3 inches above the soil some of the ants lived 2 days.

From the results of these experiments, it would seem that soil acidity would not be very useful as a means of controling or extermination such insects because in order to get an immediate or rapid killing the acidity of a soil must be increased to a point much above that which vegetable life can endure. It is, also, evident that the acidity must be uniform throughout all parts of the ant nest to effect their extermination. This was fairly well shown in the previously mentioned preliminary experiments in which the toxicity of sulfuric acid to this species of ant was being tested in cages from which the soil could not conveniently be removed and consequently the acidity was not uniform throughout it.

White Grubs.

In the series of experiments the data of which are given in Table IV, wooden cages with glass sides were used as soil containers. These cages did not prove very satisfactory and were used only for this one series of tests. In all the subsequent experiments, in which the toxicity of acid soil to white grubs was tested, flower pots three inches in diameter or jelly glasses were used as soil containers. The vents in the bottom of the pots were tightly corked, to prevent the loss of water and, especially in the case of wireworms, the escape of the insect larva. Both the pots and the tumblers were covered with small plates of glass to prevent excessive loss of moisture through evaporation. The data of these experiments are given in Table V.

The soils of the several pots of each of these experiments were prepared from the stocks of acid soils the preparation of which has been described earlier in this paper. Each series, which consisted of six or eight soils, was prepared in such a manner that their pH values formed a gradually increasing series of hydrogen-ion concentrations ranging from almost neutral to very acid as shown by the pH values in the table.

In preparing these experiments equal quantities of air-dry stocks of acid soils were placed in the various containers and made up to a moisture content which, as previously noted, was about the optimum for plant growth. By frequent weighings, and additions of water when necessary, the moisture content of each soil was kept as constant, throughout the course of the series of tests to which it belonged, as the conditions would permit.

In the series of soils the data of which are presented in Table IV, 4 or 5 grubs were placed in each container. This plan, however, did not prove satisfactory because observations could not be made upon the individual grubs and, besides, the grubs were liable to pinch each other; in some cases it was thought that the death of the grub was due to this cause rather than to any other. In most or all of the experiments the data of which are given in Table V, but one grub was placed in each container.

Observations were made, at frequent intervals, as to the presence and condition of the grub. Both soil and grub were carefully removed from the container, and put back into the

container after the observations had been made. At the same time a fresh supply of food was provided, if necessary. Each soil was thoroughly mixed before being returned to the container.

In the first series of tests, although the average longevity of the grubs in the more acid soils (those treated with sulfuric

TABLE IV.

DATA RELATING TO EXPERIMENTS ON THE TOLERANCE OF WHITE GRUBS FOR ACID SOILS.

Cage No.	Number of Grubs	Daily Water Requirement c. c.	Average pH Value	Days of Tolerance	Average Days of Tolerance
19	4	3.48	2.38	7 17 14 41	17.25
22	4	2.50	2.55	7 7 7 14	8.8
20	4	3.11	2.63	3 7 13 15	9.5
23	5	2.7	2.75	7 24 24 41 41	27.4
24	5	7.2	5.9	13 41 41 41 41	35.4
21	4	7.6	6.1	13 24 41 41	29.8

acid) was less than that of the grubs in the checks, some of the grubs in these soils lived as long as any of those in the check soils. The data relating to this series of experiments are presented in Table IV. Cages numbered 21 and 24 contained soils which had been taken from the field in which most of the grubs used in this research were collected.

The data in Table IV and V show considerable variation in the length of time the grubs lived in the various soils. How-

ever, only when the soil acidity was greater than that expressed by a pH value of 2.7 did there seem to be a decided toxicity of such soils to the white grub.

These variations in longevity can perhaps be partially explained through the fact that, since there was no means by which the temperature of the soil in the various containers could be controled, the temperature in some of the soils, at times, ran very high. This undoubtedly had a considerable effect on the grubs. In fact, several grubs were found dead during and just

TABLE V.

Data Relating to Experiments on the Tolerance of White Grubs for Acid Soils.

Pot No.	pH Value	Daily Water Requirement c. c.	Days of Tolerance 1st Grub (a)	Days of Tolerance 2nd Grub (b)
50c	1.9	0.35	2	2
50f	1.9	.....	3	2
50	2.7	5.60	28	.....
50a	2.8	7.56	45	.....
50b	2.9	8.24	28	.....
51a	3.3	7.63	29	.....
51b	3.45	8.38	23	.....
52a	4.10	14.90	45	.....
52b	4.75	13.20	47	47
54a	5.20	9.00	29	.....
53b	5.30	10.46	47	.....
53a	5.60	13.44	29	.....
54b	6.00	.....	53	.....

(a) In all the tests where the pH value was greater than 1.9, the grub was alive at the time the above notations were made, but dead or missing at the time of the next observation.

(b) The data in this column relate to cases where a second grub was placed in the soil of that pot.

following such elevations of temperature. These rises in temperature also caused a more rapid evaporation of moisture from the various soils, especially the less acid ones; these as shown in the tables, had a much greater daily moisture requirement than the more acid soils. This variation in moisture content also undoubtedly had some effect upon the grubs.

Wireworms and Cutworms.

In order to obtain an idea as to the toxicity of acid soil to some other soil insects, several larvæ of both wireworms and cutworms were placed, along with the grubs, in some of the more acid soils of the preceding white grub experiments. In

general the observations showed that acid soil is just as toxic to these insects as to the grubs and ants. When the soil was less acid than is indicated by a pH value of 2.7, little if any toxicity was noted; when the acidity was greater than that indicated by a pH of 1.9, the soils were very toxic to these larvæ.

Nesting Reactions of Ants to Acid Soils.

In order to determine whether acid soils exert any repellent or attrahent effect upon the nesting activities of these ants, a series of soils ranging in pH value from 2.0 to 6.6 were prepared. Four small jars were filled with stocks of acid soil the pH values of which were 2.0, 2.8, 6.2, and 6.6, respectively. They were then made up to equal moisture contents and placed side by side in a large glass jar. Each small jar was covered with a piece of cardboard in which there was a perforation through which the ants could enter. The chief purpose of this cover was to retard evaporation from the soil. Granulated sugar was placed on each cover to serve as food. Thirty active ants (*Formica fusca subsericea*) were placed in the outer jar, which was then covered with a tight fitting lid of window screen to prevent their escape. Daily observations were made to note which jars the ants were selecting for nesting purposes. Although they made some shallow burrows in the most acid soil, they did not form or maintain an active nest in it. In all the other jars, they built nests and, even in the soil where the pH value was 2.8, they seemed fully as active as in the less acid soil. From this it would appear that soil as acid as 2.8 pH does not exert a repellent or attrahent action upon these ants. However, soil with a pH value of 2.0 did seem to have a repellent effect upon them.

Toxicity to Insects of Soils Treated With Sulfur.

In testing the toxicity of soil treated with sulfur to insects, both grubs and ants were used. A small quantity of air-dry soil was placed in each of several small containers. The soil of each was then treated with a previously determined quantity of flour of sulfur. In all cases the sulfur was intimately mixed with the soil. After adding the same quantity of water to each soil the insects were introduced. Observations were then made from time to time as to whether soils treated in this manner exerted any insecticidal effect upon the insect in question.

From these observations deductions were made as to the toxicity to the insect of soils treated with sulfur.

Ants.

In testing the toxicity of sulfur treated soils to ants (*Formica fusca subsericea*), two series of experiments, consisting of 5 cages each, were performed. In each of these jelly glasses were used as soil containers. Equal quantities of air-dry soil, about neutral or slightly acid in reaction were placed in each tumbler. The soils were then made up to approximately the moisture content which is optimum for plant growth, and an equal number of ants were placed upon the soil of each container. Each tumbler was then partially covered with a small piece of cardboard, to prevent so rapid evaporation of the moisture.

Each tumbler was placed upon a block of wood in a large pan and surrounded with water nearly to the depth of the block, to prevent as far as possible the escape of the ants. A small quantity of granulated sugar to serve as food was placed on the block upon which the tumbler was set.

After the ants had burrowed into the soil and established nests, the soil in four of the containers in each series were treated with sulfur at the rates of 1,000, 3,000, 5,000, and 10,000 pounds per acre respectively. The other soil in each series was left untreated to serve as a check. Flour of sulfur was used in one of these series and inoculated flour of sulfur in the other.

As in the preceeding experiments, the moisture content was in each case kept fairly constant by frequent weighings and the addition of water when necessary.

Observations were made from time to time as to the activity of the ants. On March 26, 45 days after the ants had been placed in the cage, evidence of activity on their part was noted in all the soils, and it seemed to be just as great in some of the most heavily treated soils as in the checks. From these tests it would seem that sulfur does not at once exert an appreciable insecticidal effect upon this species of ant.

White Grubs.

In testing the toxicity of soil treated with sulfur to the common white grub (Phyllophaga Sp.), cage experiments similar to those in the preceding ant experiments were used. The soils of these cages were prepared from stocks of soils which had been made acid, as noted before, by treating them with solutions of sulfuric acid.

Two series of experiments (Known as Series D and Series E), each of which consisted of five soils ranging in pH value from 2.5 to 6.3, were prepared. The soil of each pot of Series D was treated with ¼ gram of sulfur to each 100 grams of air-dry soil, a quantity equivalent to 5,000 pounds per acre; that in Series E was treated with four times as much, or 1 gram, which was equivalent to 20,000 pounds per acre. One grub was placed in each pot, and food was supplied through the use of grass roots, corn kernels, or corn seedlings. These experiments were run from June 27 to July 7, or a period of ten days.

TABLE VI.

Data Relating to Experiments on the Toxicity of Sulfur-treated Soils to White Grubs.

Series D; ¼ Gram of Sulfur per Pot			Series E; 1 Gram of Sulfur per Pot		
Pot Number	Average pH of Soil	Days to Death of Grub	Pot Number	Average pH of Soil	Days to Death of Grub
50 D	2.6	2	50 E	2.4	1
51 D	4.2	(a)	51 E	3.7	(a)
52 D	5.4	(a)	52 E	4.9	(a)
53 D	5.5	10	53 E	5.3	(a)
54 D	6.3	(b)	54 E	6.2	(a)

(a) Grub living at the end of the experiment.
(b) No grub found at the time of the last observation.

Soils of varying acidities were used in these tests in order to ascertain whether the initial acidity of the soil would in any way affect sulfur so as to produce immediate insecticidal properties. The data of these experiments are presented in Table VI.

In each series the soil with the greatest initial acidity, though the same quantity of water per gram of soil had been used in its preparation, seemed much more watery than any of the other soils. In fact this soil in each series was decidely sloppy; it is thought that this condition, rather than the sulfur, caused the death of the grubs in the most acid soils.

From the table it can be seen that in the two series seven of the ten grubs lived 10 days and only one of these seven was dead at the termination of the experiment. The six living grubs were very active, even though, in some cases, the feeding conditions were not entirely satisfactory.

Since the death or disappearance of the insect could not, in any case, be ascribed to the sulfur, the conclusion was reached, that sulfur when applied to the soil in which grubs or ants are working has no direct or immediate effect which can be utilized insecticidally against these insects.

Insect Infestation of Root Crops Grown on Soils Treated With Sulfur.

Several series of plot experiments were run in the summers of 1923 and 1924 in order to find out if sulfur, when applied to soils in much the same manner as that where it is used in the control of potato scab, would in any degree effect the control of insects commonly infesting some truck garden crops. Radishes, turnips, and potatoes were used in these tests. The details and results of these experiments are given in the succeeding pages of this paper.

Radishes.

Three series of plots eight feet square were utilized in the summers of 1923 and 1924 to determine what effect sulfur treatments would have on the degree of infestation of radishes by the maggots, *Hylemyia fusciceps* and *Hylemyia brassicea*.

The experiments, the data of which are given in Tables VII and VIII, were located on a tract of lowland soil near the university. This place was chosen on account of its availability and because the soil seemed to be very fertile, and therefore a good place in which to grow root crops.

To prevent drainage from one plot to another, the plots were slightly elevated by using the soil from lanes, or shallow ditches, 3 to 4 feet wide between them. These ditches seemed effectively to take care of the surface drainage.

In 1923 the amounts of sulfur indicated in Table VII were applied uniformly to each plot, and worked into the surface of the soil by means of a garden rake before the radishes were sown. In this series of plots the seed was sown broadcast and the soil was not there-after cultivated.

The hydrogen-ion concentration of the soil of each plot was determined before the applications of sulfur were made, and at intervals in the summer until the termination of the experiment. The pH values given in the table are those determined from the samples taken at the beginning of the tests, and just before the radishes were pulled.

As shown in the table, the acidity of the soil was not, in any case, very much increased by the sulfur. Inoculated sulfur gave a slightly greater increase in acidity than uninoculated flour of sulfur.

Since the acidity of the soil of the plots in the 1923 series of tests was not increased as much as was anticipated, another

TABLE VII.

DATA RELATING TO EXPERIMENTS IN 1923 ON THE DEGREE OF MAGGOT INFESTATION OF CHINESE WHITE RADISHES ON SOILS TREATED WITH SULFUR.

Plot No.	pH June 27 (a)	Quantity of Sulfur Applied (Pounds per Acre)	Average pH August 20 (a)	Number of Radishes Pulled	Radishes Infested by Maggots	
					Number	Percent
45	7.3 7.4	200	7.1 7.3	162	82	50.61
46	7.3 7.3	500	7.1 7.2	156	66	42.31
47	7.3 7.3	Check	7.3 7.3	157	47	29.93
48	7.3 7.3	1,000	6.8 7.2	167	23	13.78
49	7.3 7.2	Check	7.3 7.3	147	20	13.61
50	7.3 7.3	500 (b)	7.2 7.4	160	18	11.25
51	7.2 7.3	2,000 (c)	6.6 6.8	74	7	9.46

(a) The upper figure for pH value in each case is for the surface (less than two inches in depth), and the lower for the sub-surface (two to four inches in depth).

(b) This plot was treated with flour of sulfur instead of inoculated sulfur.

(c) Plot 51 was seeded about two weeks later than the other plots.

series of experiments on the same plots was carried out during the summer of 1924. The results of this series of experiments are given in Table VIII.

The soil of each plot was prepared by means of a small garden plow in such a way as not to disturb the arrangement of the plots of the previous season. As indicated in the table, the same quantity of sulfur was applied to each plot, as in 1923. Plot No. 45 was not included in the series of experiments for 1924. Instead of one variety of radishes as in 1923, two

varieties were used in 1924. Instead of being broadcast the radishes were seeded in drills, thus making possible the cultivation of the surface soil while the radishes were small.

As shown in the table, the second year's treatments of sulfur, like those of the first, had little effect on the hydrogen-ion concentration. Just why these soils failed to respond more readily to sulfur treatments is not known. However, since the soil acidity was but slightly increased by the application of as much as 4,000 pounds of sulfur per acre, it was concluded that this soil was very highly buffered.

TABLE VIII.

DATA RELATING TO EXPERIMENTS IN 1924 ON THE INFESTATION BY MAGGOTS OF RADISHES GROWN ON SOIL TREATED WITH SULFUR.

Plot No.	pH Value May 8 (c)	Quantity of Sulfur per Acre (Pounds)	pH Value July 23 (c)	Variety of Radishes			
				Chinese White		Cincinnati Market	
				Radishes Pulled		Radishes Pulled	
				Number	Percent Infested	Number	Percent Infested
46	6.40	500	6.45	164	58.5	455	14.5
47	6.95	(a)	6.70	157	73.2	497	21.9
48	6.50	1,000	6.30	156	75.0	486	20.2
49	6.95	(a)	6.70	174	52.3	442	17.9
50	7.00	500 (b)	6.45	148	60.8	422	19.2
51	6.50	2,000	6.40	166	40.4	411	18.0

(a) These plots were checks and received no treatments of sulfur, but in other respects were handled just like the treated plots.

(b) Flour of sulfur was used on this plot instead of inoculated sulfur.

(c) These values were derived from determinations made upon a composite of several samples of soil, to the depth of 6 inches, taken at various places on each plot.

From the data of these two series of experiments, it is quite evident that control of root maggots, under the conditions of these tests, is not brought about by treating a soil with either flour of sulfur or inoculated sulfur.

In the summer of 1924 a third series of radish plots was arranged to test the effect of the residual acidity of a soil, which has become acid through the oxidation of elemental sulfur, upon the degree of infestation of radishes by root maggots.

Since the soils of two of the sulfur-treated plots of the 1923 series had become decidedly acid, one of each of the 5 plots of the series of 1924 was located at one end of each of the above mentioned turnip plots respectively.

The soils of these plots were prepared by means of a small garden plow, and then seeded to radishes without making any additional applications of sulfur.

The hydrogen-ion values given in Table IX are from a composite sample of soil taken from samplings made at four different places on each plot, about two weeks before the radishes were pulled.

The percentage of infestation given in the table are based on counts of from 225 to 250 radishes pulled at random from each plot. It is apparent that high soil acidity did not, in this case, bring about a control of root maggots. On the contrary, data in Table IX would seem to indicate exactly the opposite.

TABLE IX.

Data Relating to Maggot Infestation of Radishes Grown in 1924 on Soil Treated With Sulfur in 1923.

Plot No.	Pounds of Sulfur per Acre Applied in 1923, Only	pH Value in 1924 at the Time the Roots were Pulled	Percentage of Maggot Infestation
100	669	5.7	51.1
101	(a)	6.2	59.73
102	1,333	4.7	69.51
103	(a)	6.1	66.21
104	2,667	3.9	81.08

(a) These plots were checks and had received no treatments with sulfuur.

However, the writer does not think such a conclusion should be drawn until more data are available.

Since the production of H_2S has been demonstrated in composts of manure, sulfur, and soil, it was deemed advisable to arrange a series of plots and to treat the soils of the different plots with varying quantities of manure and sulfur, and after seeding them to radishes to note if sulfur, under such conditions, produced any perceptible control of root maggots.

A series of eight plots similar to those the data of which are given in Tables VII and VIII, were prepared on a tract of ground adjacent to the above mentioned plots. Sulfur and manure, as noted in Table X, were applied to the surface of each soil and worked into it by spading the plot to the depth of 6 or 8 inches.

The data in the table are based on counts of the number of radishes showing injury by maggots at the time the crop was

harvested. From the percentage of infestation of the radishes on the different plots it is very evident that the treatments of sulfur and manure did not, under the conditions of this experiment, effect a control of root maggots.

Turnips.

During the summer of 1923 six plots, each 16 by 48 feet in size, were prepared. The plots were arranged as nearly as possible so that the check plots alternated with those treated with sulfur.

TABLE X.

DATA RELATING TO MAGGOT INFESTATION OF RADISHES GROWN ON SOILS TREATED WITH MANURE AND SULFUR.

Plot No.	pH Value July 23	Quantity of Sulfur (Pounds per Acre)	Number of Radishes Pulled	Percentage of Infestation
26 (a)	6.9	500	369	85.9
27 (a)	7.0	1,000	407	81.8
28 (a)	6.95	(c)	450	80.9
29 (a)	6.9	2,000	426	72.3
30 (b)	6.9	500	425	80.7
31 (b)	6.8	1,000	452	75.0
32 (b)	6.8	(c)	579	70.8
33 (b)	6.7	2,000	410	74.4

(a) The plots thus designated in the table received an application of one bushel of manure per plot, or an amount equivalent to 9.7 tons per acre.

(b) These plots received an application of two bushels per plot.

(c) These plots were checks and were not treated with sulfur.

In preparing these plots each one was plowed as a back-furrow, thus leaving an open dead furrow between successive plots. These furrows took care of the surface drainage, thus preventing water from running from one plot to another.

After the plots had been prepared they were treated with the quantities of sulfur noted in Table XI. This sulfur was applied broadcast, as evenly as possible, and worked into the surface of the soil with a garden rake. The turnips were then seeded broadcast, care being taken to use about the same quanity of seed on each plot.

A few observations were made from time to time in the course of the experiment but the percentage of infestation given in the table are based upon counts of the number of turnips showing signs of injury by the maggots at the time they were pulled.

As can be seen from the table, there was no significant variation in the degree of infestation of the turnips on the treated as compared with the untreated plots. While the average percentage of infestation on the two check plots was less than that on the treated plots, the percentage of infestation of one of the

TABLE XI.

DATA RELATING TO MAGGOT INFESTATION OF TURNIPS GROWN IN 1923 ON PLOTS OF SOIL TREATED WITH SULFUR.

Plot	Sulfur Pounds	pH (a) Value	Number of Turnips			Turnips Russeted		Weight of Turnips Pulled	
			Pulled	Infested	Percent	Number	Percent	Total Grams	Average Grams
100	669	5.3 5.8 5.8	1321	216	16.35	205	15.52	49,305	37.32
101 (b)	.....	5.9 5.9 5.9	2372	238	10.03	0	.....	102,770	43.33
102	1333	3.5 5.4 5.7	1244	203	16.32	1088	87.46	5,550	44.50
103 (b)	.....	5.9 5.9 5.9	1107	189	17.07	0	.....	59,685	53.92
104	2669	4.1 5.1 5.9	1087	253	22.91	651	59.89	38,950	35.02
110 (c)	669	..... 5.5 5.5	672	236	35.12	265	39.43	19,155	28.50

(a) The upper number in each case is for the surface inch of soil, the second for the first 2 inches of surface soil, and the lower one for the sub-surface, (2 to 4 inches deep).

(b) These plots were checks and received no treatments with sulfur.

(c) This plot was treated with flowers of sulfur instead of inoculated sulfur.

checks was greater than that of two of the treated plots. With the exception of the plot treated with flowers of sulfur, the difference in degree of infestation on the checks and treated plots was rather small. If the data of this series of experiments indicate a tendency in any direction, they seem to show that sulfur increased rather than decreased the degree of infestation by root maggots. However, the writer does not consider the

difference in degree of infestation of the treated and untreated plots either great enough or consistent enough to warrant such a conclusion. It is, however, safe to say that treatments with sulfur did not in this case effect a control of root maggots.

The percentage of infestation on the plot treated with flowers of sulfur was considerably higher than those of either the check plots or the plots treated with inoculated sulfur. The writer has no satisfactory explanation for this difference. This plot was slightly more sandy and gravelly than the others, and this may have in some way favorably influenced the flies.

As shown in the table, the acidity of the soil of the plots treated with sulfur, expecially within two inches of the surface, was greatly increased. In general, the apparent size of the turnips on the plots treated with sulfur was slightly less than those on the check plots. The figures for the average weight of the turnips on the various plots Table XI, as well as the pictures of the turnips Plate I, Fig. A, also show this to some extent. Aside from this, only slight differences, if any, in the growth of the turnips on the several plots were noted in this season.

Potatoes.

In the spring of 1924 a tract of grass land which had been farmed several years ago was found to be somewhat heavily infested with the common white grub. Since these grubs frequently cause an appreciable amount of injury to potatoes, the writer decided to run some experiments on this tract of ground to see if inoculated sulfur had any value as a control for white grubs.

This tract of land was broken as soon as the frost was out of the ground, and the soil in proper condition to be worked. After having been properly prepared, it was divided into four plots each 66 feet long and 33 feet wide. These plots were arranged end to end, and the rows on them so planned that it was possible to cultivate from one end of the series to the other, thus facilitating their care.

When applying the sulfur and planting the potatoes it was noted that the soil of Plot 203 was somewhat heavier than that of the other plots; this plot was therefore divided and one half of it left untreated to serve as an additional check. This plot was given the number 203A.

With the exception of plots 203 and 203A, a lane 8 feet wide was left between successive plots; drainage ditches were also

TABLE XII.

DATA ON INVESTIGATIONS IN 1924 OF THE EFFECT OF SULFUR UPON THE AMOUNTS OF WHITE GRUB AND SCAB INJURIES TO POTATOES GROWN ON SOILS TREATED WITH SULFUR.

Plot No.	Sulfur Pounds per Acre	Ph Value at		Early Potatoes (Red River, Early Ohio)			Late Potatoes (Irish Cobblers)					
		Beginning	End	Yield Pounds per Plot	Percentage of Injury		Yield		Percentage of Injury			
									By Weight		By Number	
					By Grubs	By Scab	Pounds	Number	Grubs	Scab	Grubs	Scab
200	(a)	6.5 6.5	6.5 6.8	889.5	9.8	80.4	199	1496	17.6	7.54	17.3	5.4
201	200	6.5 6.5	6.2 6.2	822.0	11.2	79.0	175	1351	20.0	57.20	21.20	50.20
202	500	6.5 6.5	6.0 6.0	641.6	11.9	75.6	140	1049	27.2	56.40	24.30	50.00
203	1,000 (b)	6.5 6.5	4.4 5.5	713.6	13.5	51.3	138	964	15.0	12.90	31.10	26.00
203A	(a) (b)	6.5 6.8	6.5 6.8	801.0	10.5	63.3	184	1352	10.9	9.30	22.90	16.00

(a) These were check plots and received no sulfur treatments.

(b) Since these were just half the size of the other plots, all figures for them which indicated yield, injury, and infestation were multiplied by two, in order that their data would be comparable with that of the other plots, and the percentages were then figured.

(c) The upper figure for pH value in each case is for the surface two inches of soil and the lower one for the sub-surface (2 to 4 inches in depth).

provided at the edge of the various plots, to prevent the surface drainage of one plot from flooding the plot adjoining it, thus interfering with the tests.

The reaction of the soil was determined by colorimetric hydrogen-ion tests, both before the sulfur was applied and at the time the potatoes were dug. A few determinations of pH value were made at other times in the growing season but their results are not given in this paper.

Sulfur was applied to plots 201, 202, and 203, before the potatoes were planted, at the rate of 200, 500, and 1,000 pounds per acre, respectively. These applications were made broadcast over the surface of each plot and the sulfur was then worked into the soil by means of a wheel-hoe, in order to insure, a more rapid reaction between it and the soil.

Two varieties of potatoes were used on each plot. Six rows of the early variety and four of the late one were planted upon each plot. As both varieties were procured on the local vegetable market, the writer does not vouch for their purity. The early variety, which was called Red River Early Ohio, matured about the last of August, and the late one, which was thought to be Irsh Cobblers, matured 2 or 3 weeks later.

Since sulfur is supposed to be effective in controling scab (Phytoptera infestans) on potatoes, observations were also made as to the percentage of potatoes that showed scab lesions at the time the potatoes were dug. The data relating to both of the above types of observations are found in Table XII.

Preliminary observations were made at several times throughout the season, but the data given in the table are based on counts and weights taken at the time the potatoes were dug. As shown by the data the amount of injury caused by grubs was slightly greater on the treated plots than on the check plots. It is, therefore, concluded that sulfur, when applied as in this case, does not exert any insecticidal effect on the common white grub, even when applied at a rate as high as 1,000 pounds per acre.

Scab Infestation of Potatoes.

As stated before observations were made at the time the potatoes on the preceding plots were dug as to the amount of scab infestation. From the data, Table XII, it will be seen that there was considerable variation in the degree of scab infestation on the various plots. Although, in case of both

early and late potatoes, the plot which had received the heaviest treatments of sulfur had less scab than the other treated plots, some of the checks had decidedly less scab than the adjacent treated plots. The late potatoes on both the check plots had less scab than those on any of the sulfur treated plots. In the case of the early potatoes, though plot 203 which had received the heaviest treatment of sulfur had the least scab, the untreated end of what was originally the same plot (Plot 203A) had from 12 to 16 per cent less scab than the other two plots which had been treated with sulfur, and 17 per cent less scab than the other check which had a slightly greater amount of scab than the adjacent sulfur-treated plot.

It is evident that scab was not very effectively controlled by these sulfur treatments. On some of the plots it would seem that treatment with sulfur increased rather than decreased the degree of infestation by scab.

In most cases the infestation with scab was not so severe as to destroy the usefulness of the potatoes, and would probably not have seriously affected the salability of the crop. In this respect, however, there was no difference between the check plots and those treated.

Sulfur as a Carrier for Other Insecticides.

A preliminary test was made in which quantities of both sulfur and lime were impregnated with carbon bisulfide and nicotine sulfate, and applied to nests of ants (*Formica fusca subsericea*). Since there was no indications of insecticidal promise, this phase of the investigation was carried no farther.

Sulfur as an Insecticide for the Control of Subterranean Aphids.

As stated previously, difficulties in handling the root aphis were encountered in some preliminary tests; and as a result, work with this group of insects was practically abandoned. However, in the summer of 1924, through the courtesy and cooperation of Dr. C. R. Cutright, of the Ohio Experiment Station, an opportunity was afforded to test sulfur as a control for the black peach aphis.

The detailed procedure and results of these tests are given in Bulletin 387 of the Ohio Experiment Station. For that reason only a general account of the experiments and results will be given here.

In these tests a number of peach trees in a young orchard, which had just been planted, were treated with quantities of inoculated sulfur ranging from ½ pound to 16 pounds per tree. The sulfur was worked into the soil about the base of each tree to a depth of from 3 to 6 inches. An area of about 18 inches square, or 2¼ square feet, about each tree received treatment.

The reaction of the soil in the area treated, around each tree, was ascertained by colorimetric hydrogen-ion determinations, both before and at times in the course of the experiments. These observations were made in connection with observations of growth and of insecticidal conditions, at intervals throughout the season. The data for hydrogen-ion concentration determinations are given in Tables 2, 3, and 4, pages 220 and 221, of the above mentioned bulletin. Toward the end of the summer there was a considerable rise in the pH value of the soil around several of the trees. It was thought that the cultivation of the soil had probably mixed untreated soil with that which had been treated, and thus helped to cause a decrease in the soil acidity, as shown in the table. On October 6, when the final observations were made by Dr. Cutright, more than half of the treated trees were found dead. Living aphids were found at this time on one of the living trees. From a consideration of these facts it is evident that sulfur cannot be used as a control for the black peach aphis.

The Effect of Sulfur Treatments Upon Plant Growth.

While observations were being made on the insect infestation of root crops grown on field plots treated with sulfur, observations were also made on these same plots on the effect of treatments with sulfur upon plant growth. A general summary of these observations are given in the following paragraphs.

From Plates II and III, and from the data as to yields and weights of crops (Tables XI and XII), it is apparent that the effect of sulfur upon plant growth is variable, depending largely upon the reaction of the soil before the treatments and the extent to which the soil is buffered. None of the treatments with sulfur on the highly buffered lowland soils, the data concerning which are given in Tables VII, VIII, and X, had any appreciable effect upon the growth of radishes.

In Plate II are presented pictures of a series of radish plots which had been treated in 1923 with the quantities of sulfur

set forth in Table VII, and were treated with like amounts of sulfur in 1924, as shown in Table VIII. The stakes driven in the various plots are so marked as to indicate the approximate average height of the radishes in inches.

From the plate it is evident that the treatments with sulfur had little, if any, effect upon the top growth of these radishes, even where sulfur was applied in quantities as great as 2,000 pounds per acre for two successive years. The radishes were not weighed, but there was no apparent effect on the root growth. No russeting was apparent on any of the radishes or turnips grown on these lowland soils.

On less highly buffered upland soil, the effect of sulfur treatments upon plant growth was considerably more pronounced. On the turnip plots, the data of which are given in Table XI, such treatments did not conspicuously influence the top growth. In the following year, however, there was a very pronounced effect on the top growth of radishes and turnips on a series of plots (Table IX) arranged on one end of the turnip plots just mentioned, even though no additional sulfur treatments were made. In Plate III are shown pictures of these radish and turnip plots. The hat in the picture is on the line between the radishes and turnips on each plot. In the back ground of each picture, in the area marked *A*, is shown a portion of the previous year's turnip plots which were not included in the series of 1924. This portion of the 1923 series of plots showed very definitely that sulfur when applied to soils such as this, is very decidedly detrimental to the growth of weeds.

In the summer of 1924 Dr. D. M. DeLong planted beans on some of the unused portions of the above mentioned turnip plots and here, also, the residual effect of the sulfur treatments of 1923 was very decidedly unfavorable to the growth of plants. Even one not familiar with the location of the plots of the 1923 series, which had been treated with sulfur, could very readily point out the place where the sulfur, especially in quantities equivalent to more than 500 pounds per acre, had been applied.

The effect upon root growth was also more pronounced on the upland than upon the lowland soils. In Table XI is given the average weight of turnips on the plots treated with sulfur in 1923. Although the variation in size, as shown by the table, is not so very pronounced, it is evident that sulfur had a detrimental effect upon root growth of the turnips.

The most conspicuous effect upon the turnips of this series was that which the writer has termed "russet". In Plate I, Figure A, is shown a series of typical turnips from the various plots the data of which are given in Table XI. The darkened, chapped appearance at the top of the turnips is "russet". This so-called russet apparently occurs for the most part in approximately the surface inch of soil. As set forth in the table, russeting was more pronounced where the application of sulfur was heaviest. The turnips on the check plots showed no sign of this condition.

On the plots where 1,000 pounds of sulfur per acre was applied many of the turnips would not have been readily salable. On the radish and turnip plots, of 1924 Table IX, on the same soils no russeting was evident, although the sulfur was very detrimental to the growth of the crops.

Sulfur had no apparent influence on the top growth of potatoes, but as shown in the figures for yield, Table XII, there was a decrease in yield, in terms of both number of potatoes and pounds per plot, as the quantity of sulfur per acre was increased. In particular there was a conspicuous difference between the yields from plots 203 and 203A, which were in reality parts of the same plot as originally laid out.

From the forgoing data it is evident that in cases where the soil is not very highly buffered sulfur is likely to be detrimental to plant growth.

Summary.

1. Although SO_2 and H_2S were considerably toxic to ants when bubbled into a soil from a generator or storage tank, no evidence was found to indicate that either of these substances could be readily produced in a soil in sufficient quantity to be of insecticidal value.

2. H_2SO_3 and H_2SO_4 are toxic to ants (*Formica fusca subsericea*) when applied in sufficient quantity to a soil in which the ants have made their nests. However, the quantity necessary is greater than one might readily hope to obtain through the oxidation of sulfur in the soil.

3. Ants, white grubs, wireworms, and cutworms are able to live for a considerable time in soils with an acidity as great as that indicated by a pH value of 2.8. Such a degree of acidity would kill most of the vegetation of a soil and, even where the initial reaction was decidedly acid, would be attainable only through the use of more than 2,000 pounds of sulfur per acre.

4. Ants do not appear to be attracted to or repelled from nesting in a soil until its pH value is less than 2.8.

5. Applications of flour of sulfur and of inoculated sulfur do not seem to be toxic to ants, grubs, or root maggots; and, when applied to soils in which truck crops are grown, do not exert any appreciable insecticidal influence. Such applications, if they have any effect, seem slightly to increase maggot infestation of turnips and radishes rather than decreasing it. However, it is not thought that the data at hand is sufficient to warrant such a conclusion.

6. Sulfur did not prove effective as a control for the black peach aphis, but was very detrimental to young peach trees at Catawba Island, Ohio.

7. Sulfur was not found of any value as a carrier of insecticides such as nicotine sulfate or carbon bisulfide.

8. Sulfur was injurious to plant growth on soils which before treatment had a pH value of about 6.0 or less.

9. Scab (*Phytoptera infestans*) was not effectively controlled on potatoes, even in cases where as much as 1,000 pounds of sulfur per acre was applied.

Conclusion.

Since direct treatments of sulfur did not have any insecticidal effect upon ants or white grubs, since all of the forms of insects used tolerated a very high degree of acidity, and since in field tests sulfur showed no indications of insecticidal control, the conclusion seems inevitable that elemental sulfur can not, in light of our present knowledge, be considered to promise any value as a soil insecticide.

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EXPLANATION OF PLATES.

Plate I.

(A) Turnips from each of the plots the data for which are presented in Table XI. The rough, dark portion on the turnip, a and a' in numbers 102 and 104, is russet. Numbers 101 and 103 are from check plots and received no sulfur treatments. Numbers 100, 102, and 104 were grown in soils which had received treatments of sulfur equivalent to 669, 1,333, and 2669 pounds per acre, respectively. Number 110 is from a plot which had been treated with a quantity of flour of sulfur equivalent to 667 pounds to the acre.

(B) The cages used in the experiments on ants (Formica fusca subsericea) Tables I and III.

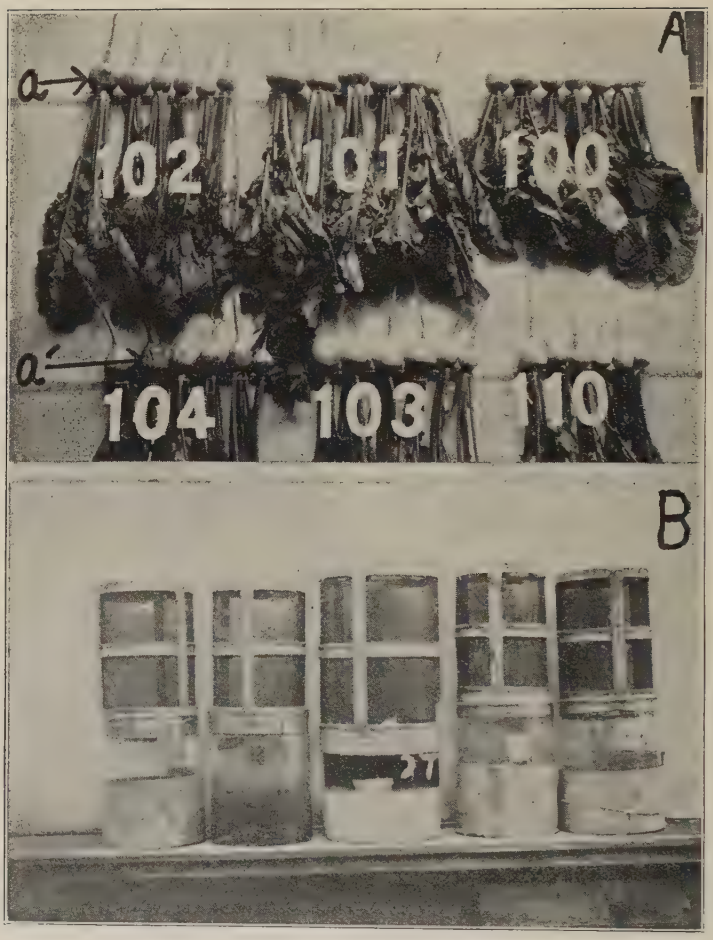
Plate II.

In this plate are shown the top growths of radishes grown on the series of plots the data concerning which are given in Tables VII and VIII. Numbers 47 and 49 received no sulfur treatments, while 46, 48, and 51 were treated with inoculated sulfur in quantities equivalent to 500, 1,000 and 2,000 pounds per acre in both 1923 and 1924. Plot 50 was treated with flour of sulfur at the rate of 500 pounds per acre. The numbers on the stakes in each plot represent in inches the height of the radishes. In each case the top of the stake is 18 inches from the surface of the ground.

Plate III.

In this plate are shown pictures of the radish plots of 1924, the data for which are presented in Table IX, and some turnip plots from which no data were tabulated. These plots were located on one end of the turnip plots of 1923, the data for which are given in Table XI. No sulfur was applied to these plots in 1924, but they show the effect of the residual soil acidity caused by the sulfur treatments of 1923. Plot 101 received no treatment with sulfur, while plots 100, 102, and 104, as shown in Table XI, were treated in 1923 with inoculated sulfur in quantities equivalent to 669, 1,333, and 2,669 pounds per acre respectively.

This section designated (*A*) in each photograph is the unused portion of the turnip plots of 1923. In this portion is shown the effect of the residual soil acidity, due to the sulfur treatments of 1923, on weed growth. In sections (*B*) and (*C*) respectively, are shown the radish and turnip plots of 1924.


A
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a'
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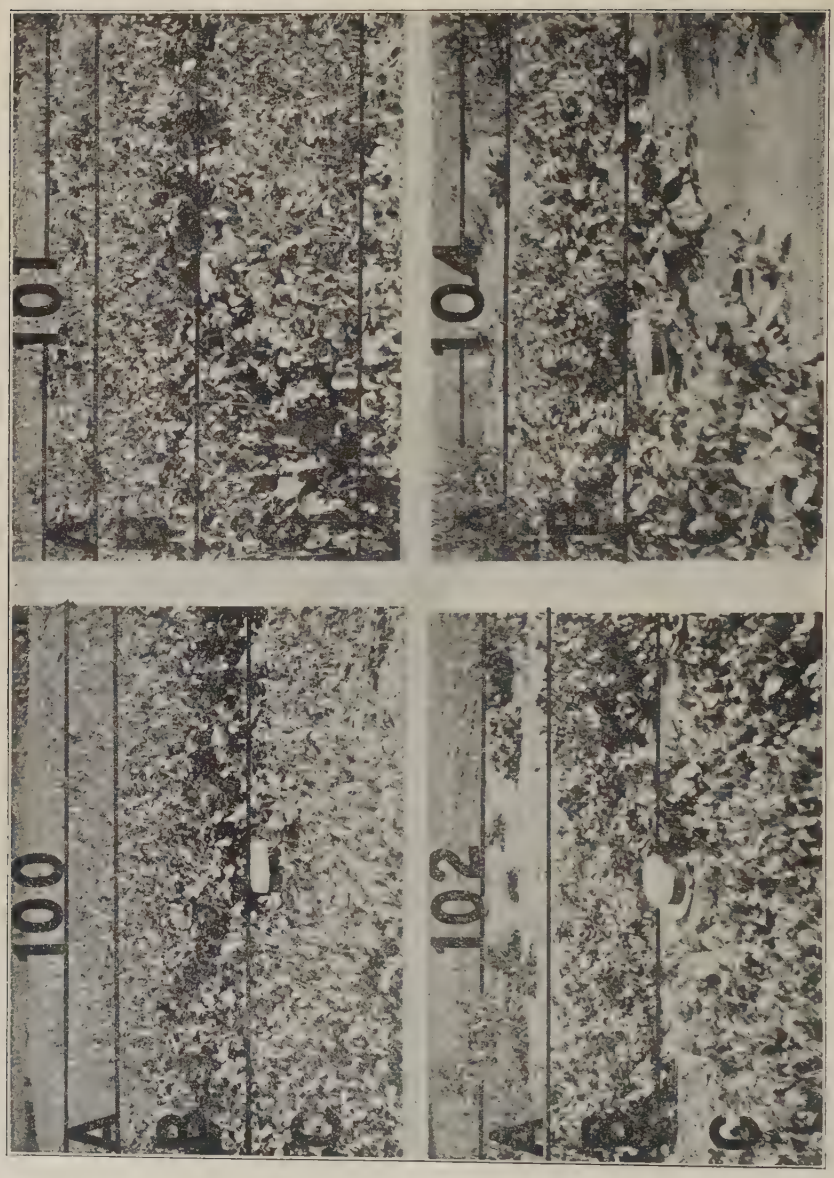
101
A
B
C
104
A
B
C
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C
102
A
B
C

THE ROOTS OF WILD RICE. ZIZANIA AQUATICA L.

E. L. STOVER,
Eastern Illinois State Teachers College.

This grass grows from Maine to Minnesota in aquatic habitats (2 and 5). It is common in marsh lands all around the Great Lakes although rapidly being destroyed in a great many places by our civilization.

The roots of this plant when it is mature, as in a great many grasses, is an adventitious root system which is submerged. The roots are rather large having a diameter as great as three or four millimeters. The root cap is of small size and extends from the tip of the root for a distance of about three millimeters. The writer was not able to find any root hairs although the growing tips of the roots are usually imbedded in the mud.

Janczewski (3) describes five types of root development as follows:

1. The root tip is composed of four primary independent tissues: the rootcap, the epidermis, the cortex and the central cylinder.

2. The root tip is composed of three primary independent tissues: the rootcap, the cortex and central cylinder.

The epidermis is derived later from the cortex.

3. The three primary tissues are the same as in the second type, but it is the calyptrogen layer developing the rootcap which gives rise to the epidermis.

4. The primary tissues are at the tip in one meristematic region.

5. The root contains only two primary tissues: the central cylinder and cortex.

From all the data that the writer has been able to obtain the grasses belong to the first two groups. Zizania aquatica belongs to the second group.

The Cortex.

In a cross section of the mature roots of wild rice the cortex is very thick and with very large air spaces (Fig. 1). The epidermal cells are small cells and in the older roots are torn and destroyed (Fig. 1) by the increase in the periphery of the root.

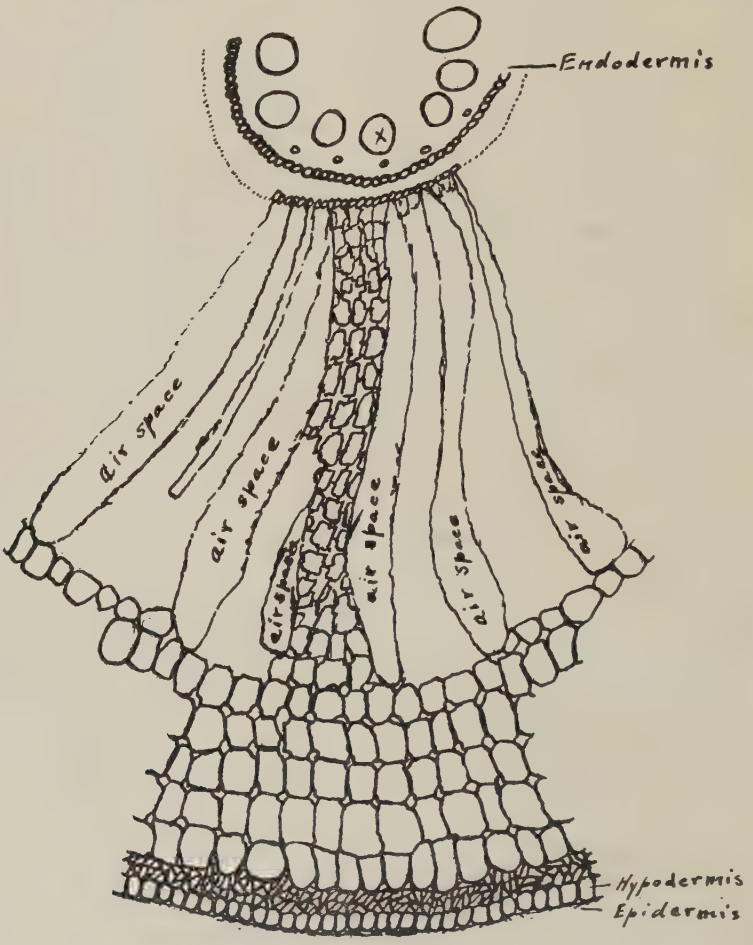


Figure 1

The hypodermis is composed of very large cells (Fig. 2) which apparently is the functioning epidermis of the older part of the root. Adjacent to the hypodermis there is a region, two or three cells in thickness (Fig. 2), which becomes lignified and functions as a mechanical tissue preventing the collapse of the aerenchyma of the cortex.

This aerenchyma of the cortex begins quite early by the pulling apart of the corners of the cortical cells. This continues with the enlargement of the cortex until the cells in the transverse section have the form of a cross (Figs. 3, 4, 5). The continuation of the enlargement of the cortex tears these cells apart completely, or actually destroys some of the cells, until the mature cortex shows radiating plates of cells with the larger cavities bordered with the torn fragments of cells (Figs. 6 and 1).

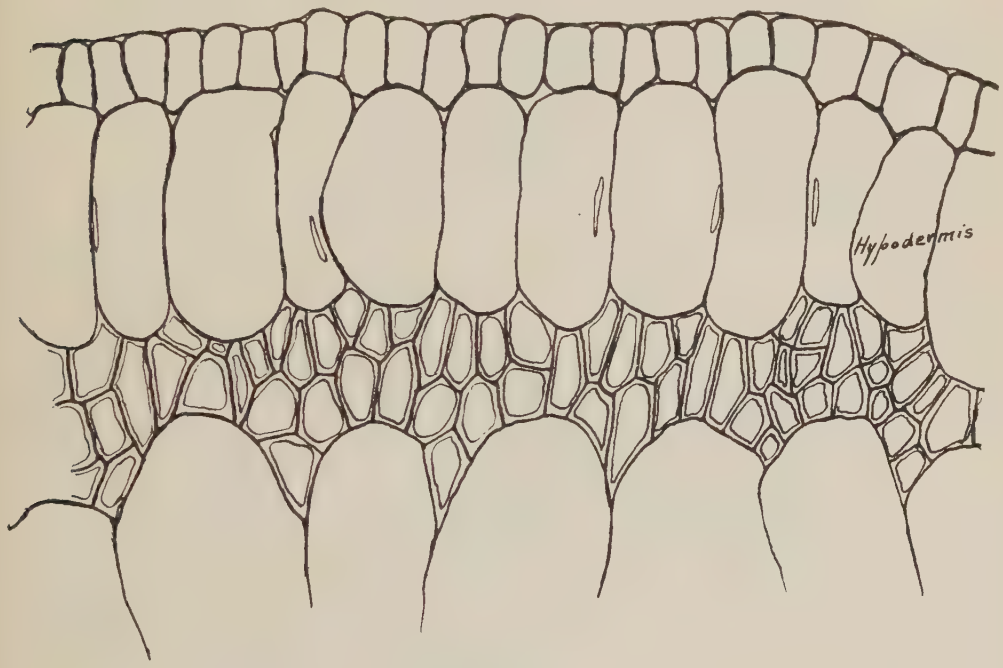


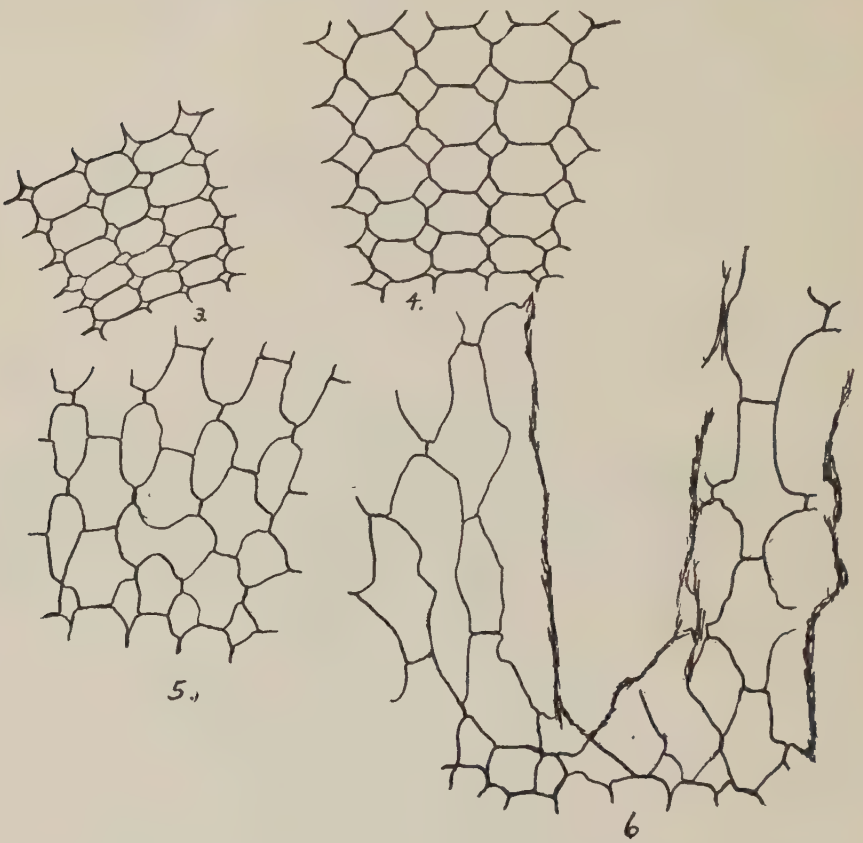
FIGURE 2

In the roots of Echinochloa walteri c. lysigenous formation of the larger cavities does not occur and is entirely schizogenous.

THE CENTRAL CYLINDER.

The central cylinder is quite easily seen in cross section at the tip of the root (Fig. 7). A very few cells from this toward the stem are the cells which finally become the so-called metaxylem vessels. They are enlarged and are easily distinguished because of this from the other cells of the central cylinder (Fig. 8). The protoxylem cells are differentiated centrifugally from these and are formed by the pericycle (Fig. 9). These cells are the first cells to have their walls thickened (Fig. 10) and because this

deposition occurs at the beginning of the region of elongation of the root, these protoxylem vessels become spiral vessels (6 and 1). Lignification begins in these cells and the surrounding cells and continues centripetally until all of the central cylinder is somewhat lignified.



FIGURES 3, 4, 5, AND 6

DIFFERENTIATION OF XYLEM.

A number of our books state that one of the differences between stems and roots is that the primary xylem cells are formed centripetally in roots. Jeffrey (4) states that it may occur in both directions. However, if we consider the morphological changes in growth from meristematic regions to be: (1) cell divisions, (2) cell enlargement and elongation, and (3) maturation (or differentiation), then it is certain that in grasses the primary xylem is centrifugal in its development although lignification occurs centripetally.

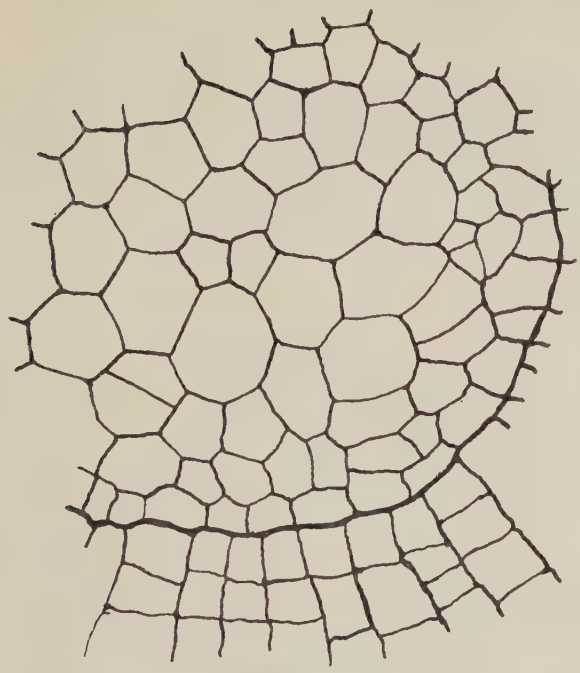


FIGURE 7

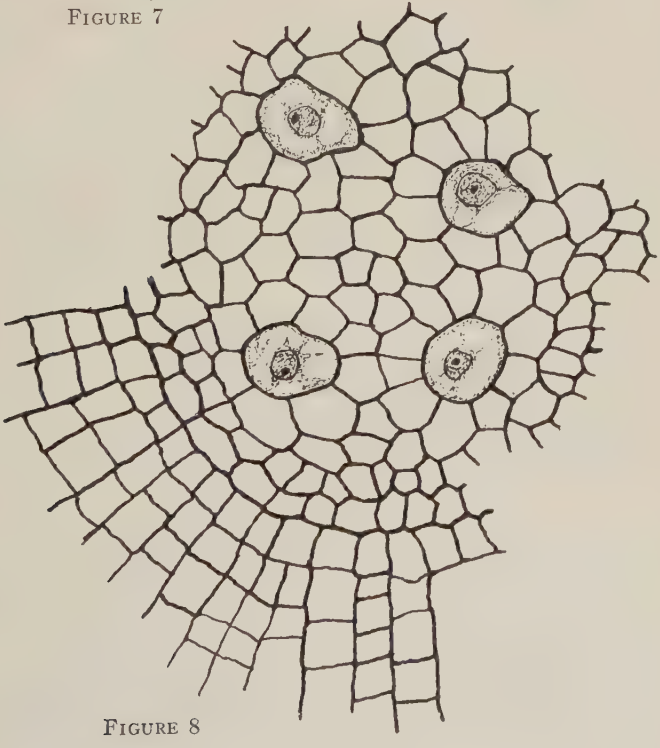


FIGURE 8

Other monocotyledonous plants examined by the writer indicate that the above development is not peculiar to the grasses as a group. The only records obtained by the writer on this point from the literature is that of Janczewski (3) in which he states that the metaxylem vessels are the first to be seen in the root tips of Fagopyrum, Pistia, Hordeum, Hydrocharis and Pisum.

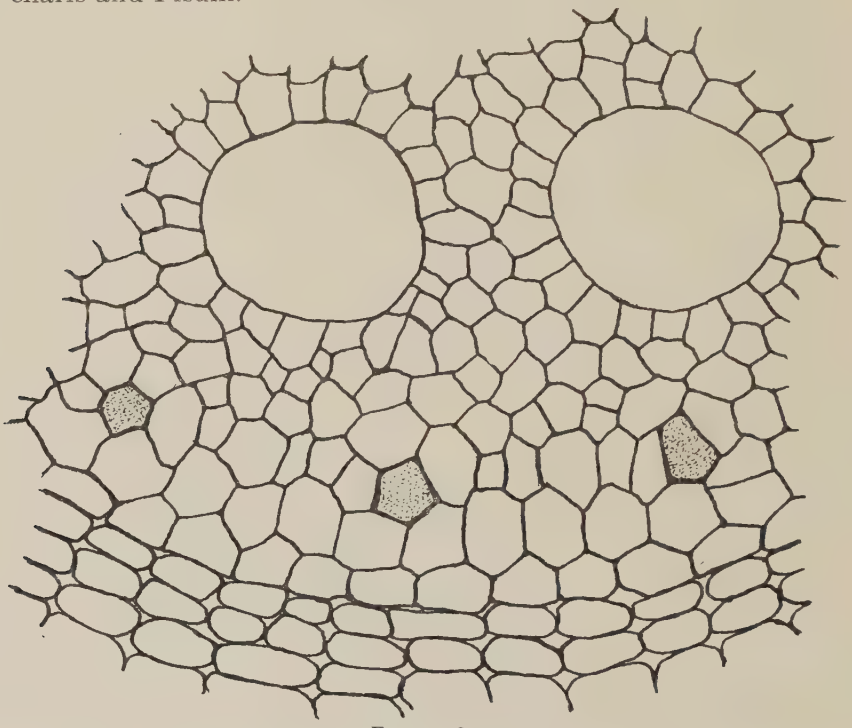


FIGURE 9

There is little doubt in the mind of the writer that the development of the primary xylem of roots is centrifugal although lignification is centripetal.

SUMMARY.

1. The formation of the air passages in the roots of Zizania are first schizogenous and finally lysigenous.

2. The epidermis is a layer of small cells, and the hypodermal cells become quite large in comparison, functioning as the epidermis because of the destruction of the epidermal cells.

3. Adjacent to the hypodermis there is a band of sclerenchyma which stiffens the outer cortex apparently preventing the

collapse of the inner cortex which is mostly aerenchyma.

4. The xylem is centrifugal in development. (a) The largest water vessels are the first to be differentiated and are nearer the center of the root. When mature they are reticulate or pitted.

(b) The second water tube is formed from the pericycle and becomes a spiral vessel, or when elongation is slight is a reticulate vessel.

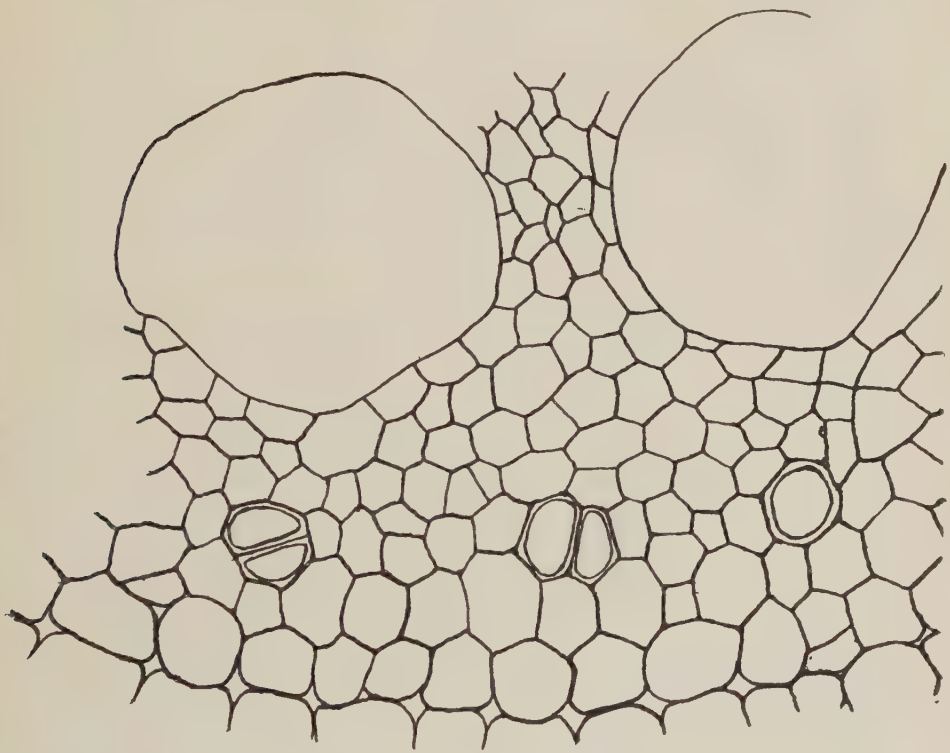


FIGURE 10

5. The second and smaller water tubes are the first lignified. Lignification then progresses toward the center of the cylinder.

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SPERMATOGENESIS IN BRANCHIPUS VERNALIS.

R. C. BAKER AND J. A. ROSOF,
Department of Anatomy, Ohio State University.

Part II.

THE PRIMARY SPERMATOCYTE.

In a previous paper, Baker and Rosof, No. 1, 1927, the general description of the testes and the behavior of the chromatin of the spermatogonia in Branchipus was reported. The salient features of the behavior of chromatin in the spermatogonia may be summarized as follows: (1) There are twenty-three chromosomes in the spematogonium of Branchipus vernalis; (2) There are eleven pairs of homologous chromosomes and one accessory; (3) In the late prophase of spermatogonia, some homologous chromosomes become paired. This pairing is more of a chance occurrence than a regular behavior in the activity of chromosomes. The present paper is limited chiefly to the description of the observations covering the chromosomal transformations of the spermatocyte of the first order.

Following the formation of the clumped, darkly staining and dense chromosomal mass of the spermatogonial telophase, a series of transformations within the spermatocyte nucleus ensue. These changes involve both karyolymph and chromatin and result in the synapsis of homologous pairs of chromosomes. One of the factors of this behavior is the mutual attraction that synaptic mates have for each other in which they exhibit an affinity greater than that shown to the other chromosomes. This does not imply that the chromosomes which are not homologous have no attraction for each other, for an attraction between unlike chromosomes is evidenced, but this relation is of a different nature from that shown by like chromosomes. The attraction of dissimilar chromosomes for each other is indicated by the end to end association which is assumed by these chromosomes in the early prophase and which has an influence on the subsequent behavior of the chromosomes.

After the last spermatogonial division, the beginning of the changes which occur in the primary spermatocyte is indicated by the diffuse attenuated condition of the chromatin which simulates a disconnected reticulum. The delicate, ill defined attenuated strands, which extend from the irregular masses of chromatin, are intermingled and reveal no apparent definite arrangement. The reticulum that characterizes this early stage is effected by the separation of the chromosomes from the dense mass of the telophase plate of the last spermatogonial division. At this time, no apparent definiteness or order can be detected in the behavior of the chromatin, but with a more detailed study the successive changes in the progression of the formation of definite chromosomes are seen, (Figs. 1 to 16 inclusive).

The relationships of strands and masses of chromatin to each other as well as the rapidity with which homologous chromosomes synapse are dependent upon the arrangement and condition of the chromosome aggregates as the reticulum formation begins.

Figure 1 is a cell taken from a different cyst than are figures 2 to 5 inclusive. This nucleus shows thirteen masses of chromatin which are somewhat peripherally arranged. Two of these chromatin masses (d and e) are well defined while the remaining masses are irregular and diffuse. These are connected by communicating strands. The strands are not definitely oriented and the number of strands extending from the more conspicuous masses of chromatin vary.

Figures 2 to 5 inclusive are taken from the same cyst. Eighteen masses of chromatin are seen in the nucleus shown in figures 2. The arrangement of the masses is similar to the preceding figure but most of the individual masses have a more definite form. At the lower part of this nucleus is a chromatin mass (f) which shows two short heavy parallel strands directed centrally from the mass. Toward the center of the nucleus there is a large conspicuous chromatin mass which is evident in the succeeding Figures 3, 4 and 5 (chromosome a). There are fifteen well defined aggregates of chromatin and eight smaller diffuse masses which give a total of twenty-three in Figure 3. Figure 4 contains seventeen masses of chromatin which in the main do not reveal such a pronounced reticulum as in the preceding Figure 3. Fourteen chromatin masses are counted in Figure 5. These masses present a more filamentous

condition than any of the previous figures. These chromatin masses lie in close proximity to each other and are disposed for the most part to one side of the nucleus. The larger aggregates in this nucleus are similar to those of the previously mentioned figures.

Figure 6 is taken from a different cyst. This nucleus shows twelve masses of chromatin which are connected by relatively thick and dark staining strands. Some of these strands resemble thin, elongate, and ill formed chromosomes. At the lower part of the nucleus a large chromatin mass (g) is visible from which extend parallel projections directed centrally. Toward the center of the nucleus six rounded bodies of chromatin connected by strands are present.

In all the figures thus far cited there is a difference in the number, size, form, and relationship of the chromatin aggregates and strands. Some of these chromatin bodies found in different nuclei show marked similarities. See chromosome (a) in Figure 2 to 5 inclusive. Referring to Figures 1 to 6, it is apparent that some of the chromosomes are similar to the telophase chromosomes of the spermatogonia previously described—in that they are well defined, round bodies which are practically free from connecting strands, (Fig. 1, d and e). The other chromatin bodies present marked degrees of variation. Some have characteristic shapes and are well defined while others are diffuse. Most of the larger masses do not present the filamentous condition which is a common characteristic of the smaller and less defined chromatin aggregates. The strands vary in length and in thickness. Some are thin and attenuated while others are better defined and extend from the chromatin bodies in a more regular manner. The chromatin masses vary in respect to their position in the strand. Some of the chromatin bodies appear as thickenings in the middle portion of the strands while others occupy distal parts.

After the irregular and contorted chromosomal configuration of the stages previously described (Figs. 1 to 6) which correspond to the preleptotene stage, a series of transformations are seen. The changes at this time result in the formation of definite chromosomal filaments which are orientated in a definite manner, (Figs. 7 to 16, inclusive). This series of changes is characterized chiefly by the transformation of chromosome bodies which are not already synapsed into definite bivalent chromosomes by parasynaptic approximation. More-

over, another process is observed which involves an orientation of the bivalent filaments into an end to end association with each other. (Figs. 12 to 16 inclusive).

This arrangement of chromosomes results in the formation of a spireme in which each of the definite bivalent chromosomal constituents are clearly definable. The spireme is not continous in the sense that the morphological identity of the individual chromosomes is lost, but it is continous in that the ends of all the bivalent chromosomes are definitely associated with each other. This end to end approximation becomes conspicuous at the beginning of loop formation which may later form a bouquet.

Only a few of the total masses of chromatin are shown in Figures 7 and 8. From these masses extend rather uniform filaments which appear to have a more definite arrangement than was seen in the preceding figures, (Figs. 1 to 5). The thick and even chromatin filaments which are joined to each other are bivalent chromosomes. The other masses and strands are not so conspicuous.

The nucleus of Figure 9 reveals the chromatin exhibiting the most uniform behavior yet shown. In the lower portion of this nucleus, univalent chromosomes are pairing side to side. The chromatin of these filaments is evenly distributed throughout their length. In the upper left part of this nucleus (h) is a well defined bivalent chromosome. A chromosome is seen at the upper right part of this nucleus (i) from which extend two similarly disposed filaments.

Only a few chromosomes are shown in Figures 10 and 11. At the left in Figure 10, one bivalent chromosome (k) is seen which has formed a loop. Beneath this loop, there are two homologous chromosomes which are attached at their extremities thus forming a somewhat oval figure. Figure 11 shows two large diffuse and irregular masses from which protrude several filaments, two of which are well defined. Aside from these two filaments this nucleus shows evidence of poor fixation.

The are 13 bodies of chromatin in Figure 12. Ten of these bodies are joined by chromatin loops and filaments. At the lower part of this Figure, there are two parallel threads of chromatin which are joined at one extremity, the other ends are obscured by a V-shaped chromosome. A bivalent chromosome (l) is seen at the upper part of this figure. Chromosome (B) of this figure is one which is synapsed at its extremities

and some distance therefrom, while its central portions are free. This partially synapsed, loop-shaped chromosome, is joined with the bivalent loop to its right and with the univalent loop to its left by intervening bodies of chromatin.

The characteristic arrangement of the bivalent chromosome loops which have joined at their extremities is seen in Figures 13, 14 and 15. These chromosomes retain their individuality although they are joined end to end. This condition is well revealed in Figure 13, which shows only a few of the total number of chromosomes. The chromosomes of Figures 14 and 15 are for the most part synapsed, and in addition, the bivalent chromosomes are joined end to end. The condition of the chromatin at this time indicates that it is not completely condensed.

It is evident that in the behavior of the chromatin already described, the following processes are involved: (1) The synapsis of homologous chromosomes; (2) The orientation of the chromosomes into an end to end association; (3) The equalization in thickness and change in form of chromosomes into definite, uniform, and elongate chromosomes.

It is inferred that the above processes are influenced by the following factors: (1) The mutual attraction that synaptic mates have for each other; (2) The attraction that unlike chromosomes have for each other which results in the end to end association of unlike chromosomes; (3) The intrinsic chromatin changes which result in the formation of definite, uniform and elongate chromosomes; (4) The differences in relationship of the chromosomes as they emerge from the last spermatogonial telophase.

The first three of the above listed factors tend towards an uniformity in behavior of the chromatin while the fourth factor interferes with the simultaneous manifestation of the previously mentioned processes. By reference to Figures 7 and 8, paper No. 1, Baker and Rosof, 1927, it is seen that in the late spermatogonial prophase there are some homologous chromosomes which are paired while others reveal no close relationship whatsoever. This relationship of chromosomes influences the behavior of chromosomes as they emerge from the last spermatogonial telophase into the prophase of the primary spermatocyte in that they remain in this paired condition during the spermatogonial division and in the early prophase of the primary spermatocyte. This is indicated by the number,

size, form, and relationship of the chromatin aggregates and strands in the different nuclei.

It is inferred that the interval and mode of synapse is dependent upon the above listed processes. The chromosomes in this species do not all synapse in the same condition, nor do they synapse at the same time. Some of the homologous chromosomes become paired in the late spermatogonial prophase, as previously stated, while others give no evidence of synapse until the beginning of bouquet formation. Some of the homologous chromosomes come into close relationship with each other, and synapse during the time when the chromatin is filamentous, while other homologous chromosomes become synapsed during the clumped condition of the chromatin. If the chromosomes synapse while in the form of filaments, then the mode of synapse is unquestionably parasynaptic, Figure 9. There is some variation in respect to the parts of the attentuated homologous chromosomes that first become approximated during synapse. Some become approximated first, at their extremities, while others at varying places along the chromosome. On the other hand, if synapse occurs during the clumped condition of chromatin, it is impossible to determine with certainty whether or not the mode of conjugation is parasynaptic or telosynaptic.

Following the last stage described (Fig. 15), which concludes the leptotene stage, the most pronounced orientation of the chromosome loops occurs. This orientation may or may not result in the formation of a bouquet. In addition to this, the chromosomes which are not already bivalent and equalized in thickness throughout become so by the time this orientation is completed. Cells in this stage reveal the ends of most of the chromosome loops oriented toward the periphery of the nucleus, some being more peripherally oriented than others. This arrangement is accomplished by the shifting of the chromosome loops which are joined end to end.

There are eight well defined bivalent loops which are associated end to end and are peripherally arranged in Figure 16. At the left side of this nucleus in close proximity to the nuclear membrane, there are seen two elongate homologous components of a bivalent chromosome (c). These homologous elements are synapsed only at their upper extremity; the remaining parts are not synapsed but show a parallel arrangement. The lower extremity of the inner most univalent

chromosome of these homologous elements already occupies an end to end relationship with a bivalent chromosome. The upper extremity of these homologous chromosomes as previously stated is synapsed, but in addition, this bivalent extremity also occupies an end to end relationship with two bivalent chromosomes. In the center of the nucleus, at a lower level than the majority of the chromosomes, a rather large and uneven chromosome is seen. This chromosome is thickened in its central portion. At the same level and to the left of this chromosome, there is a long and well defined chromosome (M) which has the form of an incomplete figure eight. Its separated extremities assume an end to end association with other chromosomes. At the upper right portion of the nucleus, two distinct chromatin bodies are seen. These bodies are nodules on the bivalent filaments which extend from them.

This figure is significant in that it practically concludes the processes which have been previously operating and marks a change in the behavior of the chromatin. Previous to this time, the processes involved are: (1) Synapses of homologous chromosomes; (2) The end to end association of dissimilar chromosomes; (3) The equalization in thickness and change in form which result in definite, uniform, bivalent, and elongate chromosomes.

Some chromosomes are seen in this figure which are not as yet completely synapsed, and some that are not equalized in thickness throughout. However, these are in the minority. This Figure as well as Figure 12 also accentuates the fact that the chromosomes do not all synapse in the same condition or at the same time. Furthermore, the general arrangement of the chromosomes in Figure 16 is indicative of the ensuing chromosome behavior.

The figures succeeding the one just described show the character of ensuing chromosomal behavior. At this time the changes which result in the formation of pachytene chromosomes may vary as follows: (1) Chromosomes may undergo complete or partial bouquet formation, and then form pachytene chromosomes or, (2) the pachytene chromosomes may be formed without undergoing a bouquet stage. Either of these two methods may be interrupted by the intervention of the so-called synezesis stage. This difference in the behavior of chromosomes at this time as just mentioned has lead to considerable confusion in the interpretation of the chromosomal changes in the primary spermatocyte.

Figures 17 to 20 show imperfect bouquets. Ten loops having their ends directed toward a common area at the periphery of the nucleus are seen in Figure 17. Above these loops, two univalent loops whose ends are joined parasynaptically are visible. A comparison of the caliber of these partially synapsed univalent chromosomes with the bivalent loops in this nucleus shows a marked difference, the bivalent loops being much thicker. Furthermore, the general contour and disposition of the univalent loops differ from the bivalent loops in that the unsynapsed chromosomes indicate a serially arranged granular appearance which is not seen in the bivalent chromosomes.

Figure 18 is comparable to Figure 17. The chromosomes (a) of Figure 18 are two bivalent chromosomes which are joined end to end and simulate the partially synapsed univalent loops of Figure 17. However, these two similar configurations of chromosomes are easily discernible on account of their differences in thickness and contour. These two figures mark the last appearance of unsynapsed chromosomes.

A different phase of bouquet formation is seen in Figures 19 and 20. Here all the chromosomes are bivalent but they are not all oriented in the same manner. Both figures show twelve chromosomes, most of which are looped. The chromosomes which are not looped have their enlarged ends either directed centrally or come into contact with some part of a loop of another chromosome. Figure 20 shows clumped and twisted chromosomes disposed toward one side of the nucleus. This may be interpreted as a fixation effect.

The complete formation of a bouquet is seen in Figures 21 and 22. In this stage the chromosomes have their ends oriented toward a restricted area at the periphery of the nucleus. Eleven loops are visible in Figure 21. The full complement of chromosomes is not seen in Figure 22 due to the plane of section.

Figures 23 to 32 show the gradual contraction of chromosomes which results in the formation of pachytene chromosomes. During this process each bivalent chromosome exhibits an individuality of its own, and the changes which each chromosome undergoes can be followed with comparative ease if synezesis does not intervene.

The arrangement of chromosomes in Figures 23 to 27 varies considerably. All of these figures reveal chromosomes which are no longer joined to each other, however, the disposition of the chromosomes varies from that of an intermingled grouping

to that of a widely separated condition. The arrangement of the chromosomes in Figure 23 is highly suggestive of a continuity of the chromosomes, but close observation reveals an intermingled grouping of ten distinct chromosomes. Figure 24 shows eleven chromosomes that reveal their free ends oriented toward the periphery of the nucleus. These chromosomes are thicker than those of the bouquet. It may be inferred that this figure is a good illustration of chromosomes which have become disengaged from the bouquet orientation. Figures 25 and 26 show a slight massing of a few chromosomes. A slightly later stage is seen in Figures 27 and 28. These chromosomes are thicker than the previously described chromosomes and resemble more closely the pachytene stage. Twelve well defined chromosomes are present in Figure 28. At the right side of this figure a U-shaped chromosome is seen whose ends are attached to the extremities of a small V-shaped chromosome lying in a different plane. The relationship of these two chromosomes is the last occurrence observed of the process which involves the end to end association of dissimilar chromosomes which was previously described.

The chromosomes of Figures 29 to 32 inclusive are in the pachytene stage. The morphological individuality of each chromosome is marked in these figures. Each chromosome has assumed a characteristic form. This may be seen by reference to Figure 31 in which there are eleven chromosomes. Furthermore, the chromosomes are no longer clumped but are distributed throughout the cell.

Following the pachytene stage the chromosomes become elongated and thickened. The beginning of this process is shown in Figure 33. This condition is well shown in Figure 34. The chromosomes in this nucleus are partially coalesced and are less distinct. Figure 35 reveals the condition succeeding that shown in Figure 34. The chromosomes in the nucleus of Figure 35 are enlarged and diffuse and at the same time, individual chromosomes are again undergoing a process of shortening. The shortened chromosomes which result are still in a diffuse condition as is seen in Figure 36. This second contraction of chromosomes may be confused with synezesis unless the continuity of this process is followed through in close stages. It is difficult to discern the exact morphological characteristics of individual chromosomes at this time. This may be attributed to the difficulty in obtaining good fixation in this stage. These

chromosomes are resolved into the chromosomes of the diakinesis stage by a rearrangement and condensation of the chromatin.

The chromosomes of the diakinesis stage are characterized by their peculiar and constant shapes. Figures 37 and 38 show a gradual transition from the more diffuse condition of Figures 35 and 26 which results from the second contraction to the distinct and characteristic chromosomes of the diakinesis stage shown in Figures 39 to 41.

A typical nucleus in diakinesis is seen in Figure 40. Twelve chromosomes are present in this figure, seven of which are more peripherally arranged than the others. In the upper right portion of this nucleus four small chromosomes are visible. Below these, a large dumbbell shaped chromosome is seen. The remaining chromosomes are median sized. The great differences in size and form of the chromosomes facilitate their identification prior to, and succeeding diakinesis. Certain chromosomes in this stage are suggestive of tetrads, but close observations fail to reveal typical tetrads. Following diakinesis, the chromosomes continue to contract and change in shape until they become more or less rounded—Figures 42 to 46. At this time, the chromosomes are going on the spindle in preparation for the ensuing meiotic division. By reference to Figures 44 to 46, it is seen that the chromosomes become joined to each other by short, darkly staining chromatin strands. These cells give a count of twelve chromosomes. A polar view of the chromosomes on the spindle is seen in Figure 47. The resulting changes which now rapidly ensue are seen in Figures 48 to 54. These include the metaphase, anaphase, and telophase stages.

From an inspection of the above figures illustrating the stages it is seen that the behavior of the X chromosome is variable. In Figure 49 it is undivided and included with the main mass of chromosomes. In Figure 50, the X chromosome is undivided and proceeds towards the pole more rapidly than do the other chromosomes. The X chromosome has divided and occupies the poles of the spindle in Figure 51. In Figure 54, the X chromosome is undivided and lags behind the divided bodies of chromatin.

The dense, darkly staining and compact mass of chromatin which characterizes the synezesis stage has been omitted in the previous description and is seen in Figures 55, 56, and 57. There is a great variation in the type of synezesis exhibited by the various nuclei. Some of the nuclei in this condition show

chromatin loops or strands extending from the main mass, while other nuclei reveal the dark mass without any projecting elements. The loops and strands which project from the main chromatin mass vary considerably. Some are attentuated and irregular while others are thick. As has been previously mentioned the chromosomes may or may not undergo synezesis. It is our observation that chromosomes may undergo synezesis at any time following the end to end association of bivalent chromosomes, Figure 16 up to the pachytene stage, Figure 29. Without synezesis the behavior of chromosomes can be followed in an orderly fashion, but by the intervention of synezesis, it is difficult to follow with certainty the orderly sequence in the behavior of the chromosomes. From the study of this material it is inferred that synezesis indicates some change in the condition of the chromosomes, which renders the chromosomes susceptible to the action of the fixatives.

The transformations of the chromosomes following the end to end association which is seen in Figure 16 are summarized as follows: (1) Chromosomes may or may not undergo bouquet formation prior to the pachytene stage; (2) These two methods may be interrupted by the intervention of synezesis; (3) The pachytene stage follows and is characterized by the shortening of chromosomes which exhibits characteristic forms. These chromosomes are scattered throughout the nucleus; (4) Following the pachytene stage, the chromosomes elongate and thicken; (5) This stage is succeeded by a thicker, shorter and more diffuse type of chromosome; (6) Following this, the chromosomes undergo a second contraction and retain for a time their diffuse condition; (7) This is succeeded by the diakinesis stage. The chromosomes of this stage are no longer diffuse but again assume a characteristic shape; (8) The ensuing phase of development reveals the chromosomes in a contracted or rounded form. At this time, some of the chromosomes are joined to each other while going on the spindle preparatory to division; (9) Meiotic division now ensues.

The chromosomal transformations in Branchipus vernalis as described in this paper compare favorably with some exceptions to the table based on Winewarter's terminology as given in Figure 262 in "The Cell in Development and Heredity," by Wilson 1925, (third edition). A general consideration of maturation in this species will be reserved for the last of this series of papers.

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EXPLANATION OF PLATES.

Plate I.

All Figures were drawn by aid of the camera lucida. Magnification of plate figures 1250 diameters. Comparisons are made as far as it is possible with the diagram, based on Winewarter's terminology in Wilson, "The Cell in Development and Heredity," Figure 262, (third edition). Figures 1 to 6 inclusive represent the preleptotene stage.

Fig. 1. This nucleus shows 13 masses of chromatin which are for the most part connected by communicating strands. The arrangement of the strands is indefinite and the number of strands extending from the more conspicuous masses varies. The reticulum, characteristic of this early stage is effected by the separation of the chromosomes from the dense mass of the telophase plate of the last spermatogonial division. This figure corresponds favorably to the resting stage (Wilson).

Figs. 2 to 5. These cells are from the same cyst. The number of chromatin masses is not constant in the various nuclei, but in no case do the masses exceed 23. The chromosome (a) is similar in form in these nuclei. The arrangement of the masses is similar to Fig. 1, but most of the masses have a definite and characteristic form. These figures correspond practically to the prochromosome stage.

Fig. 6. This nucleus shows 12 chromatin masses. Some of the chromatin strands resemble thin, elongate and ill defined chromosomes. The apparent reticulum is less pronounced. The figure is comparable to the unraveling stage.

Figs. 7 to 16. These nuclei reveal the most pronounced manifestation of the following processes: (a) Synapses of homologous chromosomes; (b) The orientation of dissimilar chromosomes into an end to end association; (c) The equalization in thickness and change in form of chromosomes into definite uniform and elongate chromosomes. These figures correspond to the leptotene stage.

Figs. 7 and 8, show only a few of the total masses of chromatin. The thick and even chromatin filaments which are joined to each other are bivalent chromosomes. The other masses and strands are not so conspicuous.

Fig. 9. The chromatin in this nucleus shows a uniform behavior. In the lower portion of the nucleus, the univalent chromosomes are pairing side by side. In the upper left portion, there is a well defined bivalent chromosome (h).

Fig. 10. At the left, in this nucleus, one bivalent chromosome (k) is seen which has formed a loop. Beneath this loop there are two homologous chromosomes which are attached at their extremities and form a somewhat oval figure.

Fig. 11. This nucleus shows two large, diffuse and irregular masses from which protrude several filaments. Two of these filaments are well defined. This nucleus shows evidence of poor fixation.

Fig. 12 shows 13 bodies of chromatin. Chromosome B of this figure is one which is synapsed at its extremities and some distance therefrom, while its central portions are free. This partially synapsed, loop-shaped chromosome is joined to a bivalent loop to the right and to a univalent loop to the left by intervening bodies of chromatin.

Figs. 13, 14, 15. The characteristic arrangement of bivalent chromosomes which have conjugated at their extremities is seen in these figures. The chromosomes retain their individuality although they are joined end to end. The condition of the chromatin in this stage indicates a diffuse state.

Fig. 16. This nucleus shows eight well defiined bivalent loops which are associated end to end and are peripherally arranged. Chromosomes C shows two elongate and homologous components of a bivalent chromosome. These homologous elements are synapsed only at their upper extremity. The remaining parts are not synapsed but show a parallel arrangement. This figure is significant in that it marks a change in the behavior of chromatin, and may be interpreted as a transition stage between leptotene and post leptotene stages.

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Plate II.

Figs. 17 to 28 inclusive show the character of the chromosome behavior prior to the pachytene stage without the intervention of synezesis.

Figs. 17 to 20. These nuclei show imperfect bouquets.

Fig. 17. Above the loops, two univalent chromosomes are seen, the ends of which are joined parasynaptically.

Fig. 18. Chromosome A of this figure represents two bivalent chromosomes which are joined end to end and simulate the partially synapsed univalent loops of Fig. 17. It is inferred that Figures 17 and 18 may be stages prior to complete bouquet formation or that they may form pachytene chromosomes without undergoing bouquet formation.

Fig. 19 and 20. These nuclei show a different type of bouquet formation. Here, the chromosomes are bivalent but they are not all arranged in the same manner. Both figures show 12 chromosomes most of which are looped. The chromosomes which are not looped have their enlarged ends either directed centrally or come into contact with some part of a loop of another chromosome. The following interpretations may be placed on these two figures. (a) They may be stages just prior to complete bouquet formation. (b) They may immediately succeed bouquet formation, (c) They may form pachytene chromosomes without forming complete bouquets, (d) These stages may mark the intervention of synezesis.

Figs. 21 and 22 show complete and typical bouquets. Eleven bivalent loops are visible in Fig. 21, and only 8 loops visible in Fig. 22. In no case are there more than 12 loops.

Figs. 23 to 29. These nuclei show chromosomes following the conditions mentioned above and just prior to the pachytene stage.

Fig. 23. The arrangement of chromosomes is highly suggestive of their continuity. Ten separate chromosomes are intermingled which suggests this continuous condition.

Fig. 24. This nucleus shows 11 chromosomes that reveal their free ends oriented toward the periphery of the nucleus. It may be inferred that this figure is a good illustration of chromosomes that have become disengaged from the bouquet orientation.

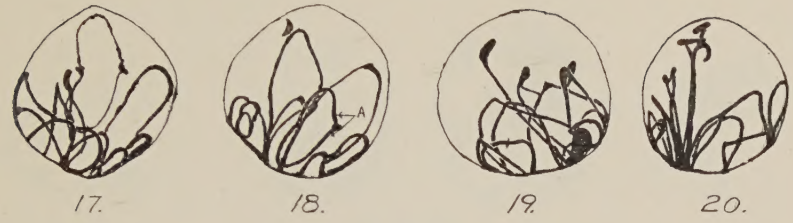
Figs. 25 and 26. These nuclei show a slight massing of a few chromosomes.

Figs. 27 and 28. These nuclei are in a later stage than any of the previous figures. These chromosomes are thicker than the previously described chromosomes and resemble more closely the pachytene stage.

Fig. 28 shows 12 well defined chromosomes. At the right side of this figure a U-shaped chromosome is seen whose ends are attached to the extremities of a small V-shaped chromosome.

Fig. 29 to 32 inclusive show nuclei in the pachytene stage. The total number of chromosomes is not present in these nuclei due to the plane of the section. However, a count of chromosomes show that in no case does the chromosome number exceed twelve.

The morphological individuality of the chromosomes in these nuclei is marked. Each chromosome has a characteristic form. The chromosomes are no longer clumped but are distributed throughout the cell.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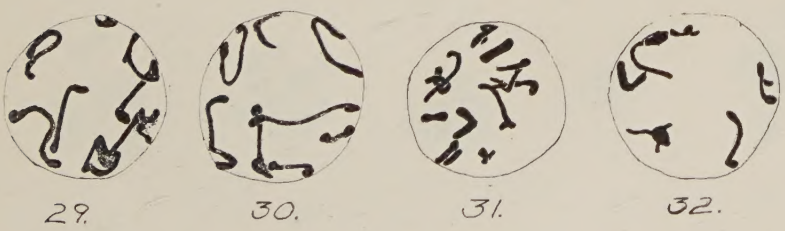
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PLATE III.

Fig. 33. This nucleus contains 12 chromosomes, some of which are elongating and thickening.

Fig. 34. The chromosomes in this nucleus are elongated and thickened. Some of the chromosomes are partially coalesced and are less distinct.

Fig. 35. The chromosomes in this stage are thickened, enlarged and diffuse, but at the same time the individual chromosomes are again undergoing a process of shortening. This corresponds to the diffuse stage.

Fig. 36. The chromosomes in this nucleus are shortened and are still in a diffuse condition. This second contraction of chromosomes may be confused with synezesis unless close stages are followed. This corresponds to the second contraction stage.

Figs. 37 and 38. These two nuclei show a gradual transition from the more diffuse condition resulting from the second contraction to the distinct and characteristic chromosomes of the diakinesis stage.

Figs. 39 to 41. These nuclei show characteristic chromosomes of the diakinesis stage.

Figs. 42 to 46 show the condition of chromosomes following diakinesis. The chromosomes at this time continue to contract and change in shape until they become more or less rounded. In Figures 42 and 43 the nuclear membrane is present. The nuclear membrane has disappeared in Figures 44, 45, 46. The chromosomes of these nuclei become joined to each other by short and darkly staining chromatin strands. These cells give a count of 12 chromosomes. In some of these figures, the centrosomes are visible.

Fig. 47. This figure is a polar view of the chromosomes on the spindle. In this figure as well as in the succeeding figures the accessory chromosome is indicated by the letter X.

Fig. 48. A profile view of a spindle.

Fig. 49. Metaphase.

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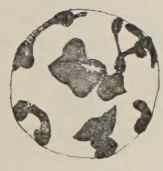

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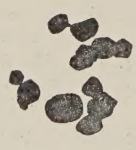

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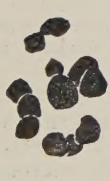

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49.

PLATE IV.

Fig. 50. A metaphase showing the undivided accessory chromosome proceeding in advance of the main mass of chromatin.

Fig. 51. The divided X chromosome occupies the poles of the spindle.

Fig. 52. Early anaphase. The X chromosome is undivided and is proceeding toward the pole with the main mass of chromatin.

Fig. 53. Late anaphase.

Fig. 54. Telophase showing the X chromosome undivided and lagging behind the divided bodies of chromatin.

Figs. 55, 56, and 57. These nuclei are in synezesis. These figures were placed at the end of the series because without synezesis the behavior of chromatin can be followed in an orderly fashion, but with the intervention of synezesis it is difficult to follow with certainty the orderly sequence. The chromosomes may undergo synezesis at any time following the end to end association of bivalent chromosomes up to the pachytene stage. The chromatin in these figures is disposed to one side of the nucleus and is arranged in a dense, darkly staining and compact mass.

Fig. 55. This nucleus shows two filaments which extend from the synezesis mass.

Fig. 56. Several irregular strands extend from the synezesis mass in this nucleus. These synezesis masses resemble closely contracted bouquets.

Fig. 57. Three distinct loops and two pronounced and elongate chromosomes are seen extending from the synezesis mass.

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